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*6,679 volunteer abstracts, 19 symposium and workshop abstracts, 2 special lecture abstracts.
1987 PROGRAM COMMITTEE

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Montreal General Hospital

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University of Iowa College of Medicine
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W.W. Vale No abstract

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98. Selective Attention and Its Neural Basis.  
Chair ed by: M. Mishkin No abstract

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**Presidential Special Lecture**— 11:30 a.m.

157. Cortical Computational Maps for Auditory Imaging. N. Suga | No abstract

**Symposia**— 1:00 p.m.

158. The Role of Nectins (Cell Binding Molecules) in Neural Development. *Chaired by:* L. Glaser | 567
159. Structural Computer Simulations in Developmental and Systems Neurobiology. *Chaired by:* J.M. Bower | 567

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215. Excitatory Synaptic Transmission in the Hippocampus. C.F. Stevens | 782

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216. Molecular Basis of Axonal Transport—Kinesin and Other Transport Proteins. T.S. Reese | No abstract

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**SUNDAY**

**Presidential Special Lecture—11:30 a.m.**

395. Perspectives on Computational Neuroscience.

T.J. Sejnowski

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**Symposium—1:00 p.m.**

396. Neuronal Serotonin Receptors.

*Chair ed by: J.M. Palacios and B.P. Richardson*

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**Workshop—1:00 p.m.**

397. New Directions in Mammalian CNS *in vitro*: Beyond the Slice. *Chair ed by: K.D. Walton*
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Theme G: Motor Systems and Sensorimotor Integration
### Theme H: Structure and Function of the CNS

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### Theme I: Neural Basis of Behavior

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In the present experiments we used drugs with specific effects on noradrenergic transmission as probes to identify some of the synaptic sites that might be affected by early malnutrition. Pineal glands were obtained from 7 day-old albino rats whose mothers were fed isocaloric normal (8%) or low-protein diets from the time of conception. Pineal glands were removed surgically under sterane and were incubated in vitro with the indirect acting sympathiomimetic drug, d-amphetamine sulfate, to assess possible malnutrition-induced postnatal changes in sympathetic noradrenergic nerve function. Changes in NAT activity were measured 4 hr later, using the method of Deguchi and Axelrod (1972). Amphetamine-induced elevations in pineal gland NAT activity were similar in both groups. Although early malnutrition may have few effects on the functioning of sympathetic noradrenergic synapses that control NAT activity, it now seems likely that it could delay the maturation of retinal and/or brain mechanisms important for mediating light-induced changes in pineal gland hormone biochemistry in developing animals.

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The development of fetal "behavior" is being heavily investigated in humans and other animals. In humans, fetuses are being studied in utero (ultrasoundography), while rat research externalizes the fetus with maintenance of placental attachment to the mother. The method is showing that externalization of the fetal rat has a behavioral complexity displayed by even very young fetuses in terms of "spontaneous" and "stimulation-induced" movement patterns. In the present investigation, fetal rats were completely delivered from their mothers (i.e., premature delivery) and their "spontaneous" behaviors were studied over an 8 hour period. Premature, female albino rats were killed (cervical fracture) and a laparotomy was performed to deliver the premature pups at 18.5, 19.5 and 20.5 days estimated gestational age (EGA). The pups were quickly freed of the uterus and amnion, and gently brushed to stimulate breathing. Vaginally delivered pups (21.5 EGA), and preterm pups, were incubated at 32-33°C. One hour after the onset of regular respiration, pups were studied for "spontaneous" movement (head, mouth, forelimbs, hindlimbs, trunk). Movement studies were repeated 7 times over 7 hours.

Movement rates of premature pups ranged from 100% at EGA-18.5 days (no spontaneous movement) to 0% at EGA-20.5 days. Body weight, length, brain weight, and cerebral width and length increased with age. Pups from 19.5 to 21.5 days EGA were all mobile. Pups at 18.5 days EGA differed from the others (e.g., lower total movement, lower percent "spontaneous" movement). In direct contrast to the outcome of stress applied randomly throughout pregnancy.


Effects of early malnutrition on light-dependent changes in rat pineal gland biochemistry. L.D. Little, R. Smart, J. L. Smart, J. L. Smart and J. L. Smart*. Department of Psychology, University of California, Santa Barbara, CA 93106.

Impaired physiology and behavior caused by early malnutrition may result from temporary or permanent impairments in synaptic function, but the lack of an appropriate animal model has made it difficult to precisely characterize these impairments. In the present study, we utilized a maturation paradigm where activity was maximal at night and lowest during the day. Brief nocturnal light exposure rapidly suppresses adult rat NAT activity via a retinal-brain-regional corticoadrenal nervous-system circuit (Klein and Weller, 1972). Nocturnal light-induced suppression of NAT activity cannot be detected until 6 days postnatal, when retinal and/or neural mechanisms sensitive to light first begin to function (Bronstein et al., 1980). Early malnutrition delays the maturation of this response by 3-4 days (Nast et al., 1986).

In the present experiments we used drugs with specific effects on noradrenergic transmission as probes to identify some of the synaptic sites that might be affected by early malnutrition. Pineal glands were obtained from 7 day-old albino rats whose mothers were fed isocaloric normal (8%) or low-protein diets from the time of conception. Pineal glands were removed surgically under sterane and were incubated in vitro with the indirect acting sympathiomimetic drug, d-amphetamine sulfate, to assess possible malnutrition-induced postnatal changes in sympathetic noradrenergic nerve function. Changes in NAT activity were measured 4 hr later, using the method of Deguchi and Axelrod (1972). Amphetamine-induced elevations in pineal gland NAT activity were similar in both groups. Although early malnutrition may have few effects on the functioning of sympathetic noradrenergic synapses that control NAT activity, it now seems likely that it could delay the maturation of retinal and/or brain mechanisms important for mediating light-induced changes in pineal gland hormone biochemistry in developing animals.

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416.13  EFFECTS OF SLEEP/WAKING STATES ON RECURRENT INHIBITION IN DENTATE GYRUS. K. Austin, J. Bronzino and P.J. Morrow (SPONSOR: J. McKeeney). Wayne State University, Wayne, MI 48154 and Department of Engineering, Trinity College, Hartford, Conn. 06106.

We previously showed that prenatal protein malnutrition affects long-term plasticity (long-term potentiation) in the dentate gyrus (Dev. Brain Res. 29:267-273, 1986). There is also evidence that transmamil transmission through the hippocampal trisynaptic circuit is vigilance state dependent (J. Neurophysiol. 41:716-732, 1978). The possibility that both malnutrition and vigilance state may influence activity in hippocampal circuits by modification of recurrent inhibitory processes is currently under investigation. In the present study we examined the effects of sleeping/waking states on recurrent inhibition in the dentate gyrus in order to determine the role of inhibitory interneurons in the vigilance state modulation of evoked extracellular field potentials. Paired-pulse tests were carried out during each vigilance state (active waking, immobile waking, slow-wave sleep and REM sleep). The paired pulse technique is a useful tool which allows us to monitor the degree of recurrent inhibition in effect in the dentate gyrus. We first determined the stimulus-response relationship and found that a population spike of maximum could be obtained using a stimulus of 600 μA by 100 μsec. Using this intensity, paired pulse tests were performed during each vigilance state using interpulse intervals of 30 and 40 msec. Population spike amplitudes from the first evoked waveform were group averaged according to vigilance state and plotted and analyzed using the Bonferroni T-test. The population spike amplitudes during immobile waking were found to be significantly smaller than during the other vigilance states (p<0.05). This is in line with earlier findings by Winson and Abzug (1978) who showed that population spikes in the dentate gyrus are smaller during slow-wave sleep than during immobile waking. Using paired pulse procedures we then showed that recurrent inhibition is itself modified as a function of vigilance state. We found that recurrent inhibition was signific mechanical more effective in inhibiting the second spike 30 msec later during slow-wave sleep and immobile waking than during REM sleep and active waking. This data indicates that recurrent inhibitory processes in the dentate gyrus are vigilance state dependent and suggests that activity in the dentate gyrus is an important role in the short-term, vigilance state modulated plasticity. Experiments in progress seek to further establish how recurrent inhibition changes as a function of vigilance states and whether these short-term plastic responses are also altered by prenatal protein malnutrition. Changes in the efficacy of neuronal transmission at different loci along the trisynaptic circuit are also being examined. (Supported by NIH grants HD-0534-13 and HD-23338-01).

416.14  HYBRID VIGOR AND MATERNAL ENVIRONMENT EFFECTS ON MOUSE BRAIN SIZE. B. Balun-Fleming*, D. Kohlstein, and J.M. Lassalle. (SPONSOR: R. Bertrand, Dept. of Psychology and Neuroscience, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1 (D.W. and B.R-B) and Lab. d’Ethologie et de Physiopathologie, Université de Tours, 37200 Tours, France (J.M.L.).) A combination of ovarian grafting and surrogate fostering was employed with BALB/c-ad (B), C57BL/6J (C), and their reciprocal F1 hybrids (BC and CB) such that each of these 4 genotypes experienced a grafted prenatal environment (1 or F) and was fostered at birth to an ungrafted littermate of the same genotype (1 or F). At 100 days of age, performance on the Morris water maze and discrimination (p<0.05) and perceptual and brain extraction at 100 days of age. The 16 combinations of genotype (G), prenatal (B), and postnatal (F) maternal environments resulted in a total of 337 mice with an average litter size of 100 days, respectively.

After adjusting for litter size effects, the 100-day brain weights were about 15 mg heavier for strain B than C and about 20 mg heavier for the hybrids than for the average of the inbreds. Animals which experienced an internal environment of strain F had brains which were about 15 mg heavier than those which experienced the inbred (I) postnatal environment (see diagram). There were no prenatal maternal environment effects on adult brain weight.


The ability of the 21-aminosteroid U-74006F (21-[4-(2,6-di-l-pyrrolidinyl-4-pyrimidinyl)-1-aminostereoidmonomethane sulfonate) inhibitor of CNS tissue lipid peroxidation (dipyrromethenyl-16-methyl-3-20-dione, mononethane sulfonate), a potent inhibitor of iron-dependent lipid peroxidation, to animals with a sample of classical active brain hyperfusion following either subarachnoid hemorrhage (SAH) or a brief (5-minute) episode of global brain ischemia was examined in anesthetized cats. SAH was experimentally produced by injection of 0.3 ml/kg of unheparinized autologous blood into the cisterna magna after prior withdrawal of an equivalent volume of cerebrospinal fluid. In animals that received an iv. dose of vehicle at 30 minutes post-SAH, there was a progressive decline in cisternal nuclear blood flow (CNBF), -50% by 6 hours and increases in intra-cerebral pressure (ICP) by 3.5-mm Hg by 3 hours. In comparison, in cats that received a 1 mg/kg iv. dose of U-74006F at 30 minutes after SAH, there was a complete prevention of the fall in CNBF and a significant attenuation of the rise in ICP. Furthermore, the drug reduced a concomitant fall in the mean arterial blood pressure. While not as effective as the 1 mg/kg iv. dose, a 0.1 mg/kg dose also significantly attenuated the post-SAH fall in CNBF.

In a second set of experiments, cortical blood flow (CBF) returned to normal or above immediately following a 5-minute episode of total brain ischemia (tourniquet-induced). Thereafter, CBF fell progressively to a level 71% below normal by 3 hours post-ischemia. In contrast, in cats that received a 1 mg/kg iv. dose of U-74006F at 15 minutes after the ischemia episode, CBF remained significantly above that seen in the vehicle-treated animals. At 3 hours, CBF was 80% of control (p<0.05 compared to vehicle treatment). In addition, U-74006F treatment significantly improved post-ischemic maintenance of blood pressure, recovery of the somatosensory-evoked potential, and the degree of post-ischemic reduction in arterial blood acids.

These results support the view that microvascular lipid peroxidation is involved in both post-ischemic and post-hemorrhagic brain hyperperfusion and suggest that the 21-aminosteroid U-74006F may be useful in the early treatment of ischemic or hemorrhagic stroke.


The possible protective effects of U74006F, a novel 21-aminosteroid (21-[4-(2,6-di-l-pyrrolidinyl-4-pyrimidinyl)-1-aminostereoid-16a-(4-(11)-trime-3-20-dione, mononethane sulfonate)) inhibitor of CNS tissue lipid peroxidation (dipyrromethenyl-16-methyl-3-20-dione, mononethane sulfonate) found in brain following a 3 hr. period of unilateral carotid occlusion in mongolian gerbils, was examined. Male Mongolian gerbils (55-65 g) received two i.p. injections of vehicle or U74006F (3 or 10 mg/kg). The first injection was given 10 min. before carotid occlusion and the second was administered at the end of the 3 hr. carotid occlusion. Only those gerbils showing positive clinical signs of brain ischemia by 1 hr. after occlusion were included. All experiments were carried out blind.

In a first series of experiments, vehicle treated gerbils displayed a 60.9% survival at 24 hrs. post-ischemia which dropped to 34.8% at 48 hrs. In contrast, the 10 mg/kg U74006F treated group showed an 86.7% survival at 24 hrs. (p<0.01 vs vehicle) and an 80.0% survival at 48 hrs. (p<0.01). The 3 mg/kg treated animals displayed only a slight improvement in survival at 24 hrs. at 78.4%. There was no difference in vehicle group which was 68 hrs. at 28.6.

In a second series, a comparison of neuronal densities was made in the hippocampal CA1 subfield and medial and lateral cerebral cortex in surviving animals at 24 hrs. after unilateral carotid occlusion. Comparing the cell densities in the ischemic hemisphere to those in the contralateral non-ischemic hemisphere revealed a significantly better neuronal preservation as a result of U74006F (10 mg/kg i.p. 1.5 hr. treatment) in vehicle animals, the mean neuronal densities, in relation to the non-ischemic hemisphere, was 26% lower in the CA1 subfield, 20.4% in the medial cortex, and 31.9% in the lateral cortex. In U74006F treated gerbils, the mean densities were 52.0% in the CA1 subfield (p<0.001 vs medial cortex) and 48.9% in the lateral cortex (p<0.002).

These results show that U74006F improves post-ischemic survival and attenuate neuronal necrosis in a severe brain ischemia model and provide pharmacological support for an important role of oxygen radical-induced LP in the pathophysiology of brain ischemia followed by reperfusion.

One mechanism theorized to underlie the degenerative sequelae to a major stroke is the appearance of oxidative free radicals accompanying aberrant cellular metabolism. Free radicals can irreparably damage cell membranes via lipid peroxidation (LP). To evaluate the role of LP in stroke pathophysiology, we have determined the effects of treating experimental stroke with U-74006F (2,6-dimethyl-pyridyl-4-pyrimidinyl)-1-piperazinyl-16a-methylpregn-5,14,16-triene-3,20-dione, mono-methane sulfonate. In various in vitro assays using brain homogenates, U-74006F has been found to be a potent and effective inhibitor of iron-dependent LP. With a 50% inhibitory concentration of 18 μM, the potency of U-74006F approaches that of α-tocopherol. In contrast to a-tocopherol, however, U-74006F may be given intravenously and is rapidly taken up by neural tissue. One-hour unilateral occlusion of the middle cerebral artery in the cat resulted in massive ipsilateral lesions measured one week later by classical histology and quantitative 2-deoxyglucose autoradiography. Lesions were characterized by large infarct areas of reduced metabolic rates surrounded by a penumbra of enhanced metabolic activity. Spectral analysis revealed that lesion sides had increased numbers of pixels with metabolic rates below 11 or above 30 umole glucose/100g brain/min. Post-occlusion treatment of cats with multiple i.v. injections of U-74006F (1 mg/kg 15 min after reflow, and 0.35 mg/kg 2, 6, and 12 hr later) resulted in dramatic reductions in lesion sizes, mainly through reductions in metabolically depressed infarct areas. Highly significant reductions were noted in lesion volumes measured by spectral analysis or by tracing lesion areas. Lesion area in sections showing maximal metabolic damage were reduced by 76% in the cortex and 64% in the caudate. Lesion volumes were estimated to be reduced by 81% in both cortex and caudate. It is concluded that (1) LP may represent a crucial post-stroke step underlying stroke pathophysiology, and (2) U-74006F may represent a new and significant therapeutic entity for treatment of this debilitating condition.

417.4 EFFECTS OF HYPOXIA ON RESPONSES TO ACIDIC AMINO ACIDS IN THE IN VITRO HIPPOCAMPAL SLICE R.K. Rader*, T.B. Lasthorn, F.S. Lipton*, Gleave Research & Development, Chesterfield, No. 63598; Dept. of Physiology, Univ. of Wisconsin, Madison, WI. 53706.

Hypoxia can produce neuronal damage in vivo and in vitro. During hypoxia in the in vitro hippocampal slice, synaptic responses are abolished. This could be due to presynaptic or postsynaptic factors. In order to determine the relative importance of pre- and post-synaptic mechanisms we have recorded responses to acidic amino acids applied by pressure ejection, before and during hypoxia. Rat hippocampal slices were submerged in continually flowing artificial cerebrospinal fluid (ACSF) (1.5-2 at/min) containing 95% O2:5% CO2. Extracellular population EPSPs were recorded in stratum radiatum of CA1 in response to stimulation of the Schaffer collateral/commisural fiber. Extracellular focal potentials (FP) were induced by pressure ejection of the NMDA-type acidic amino acid agonist N-Methyl-D-aspartic acid (NMDA:100μM) and L-homocysteic acid (L-HCA:500μM) and the non-NMDA agonist willardine (100μM). Agonists were ejected into stratum radiatum of CA1 within 100μm of the recording electrode. Hypoxia was induced by replacing the ACSF containing 95% O2:5% CO2 with ACSF containing 95% N2:5% CO2. Within 2-4 min of replacing O2 with N2, the population EPSP was abolished. In contrast, focal potentials induced by any of the agonists persisted for at least 30 min (the longest period of hypoxia studied). Focal potentials induced by NMDA, L-HCA, and willardine, were reduced to 66.3±6.0%, 51.6±5.6%, and 64.3±9.0% (Mean ± SEM) of control, respectively. The results indicate that, since transmitter release is more sensitive to hypoxia than postsynaptic receptors, postsynaptic responsiveness remains even during prolonged hypoxia in vitro.


Several authors reported that stable analogues of adenosine are potent inhibitors of glutamate transmission in the brain, and that AI receptors for these compounds are particularly dense in the ischemia vulnerable regions. We have demonstrated previously that cyclohexyl adenosine (CHA) injected into the ventricles of the gerbil brain affects both qualitative and quantitative neuronal protection in the cortex, striatum and hippocampus. In the present study the impact of a single I.P. injection of CHA upon survival of severe ischemia was investigated. 57 male gerbils, 70-80g, were anesthetized with 0.5% Halothane, 33% N2O and 72 O2. Both carotid arteries were occluded with microvascular clips for 30 min resulting in forebrain ischemia. During the entire procedure blood pressure, blood O2 concentration and cardiac rate were monitored noninvasively. The body temperature was held at 36.5-37.5°C. CHA in 102 ethanol was injected (2 mg/kg) 5 min after ischemia. The controls were injected with the vehicle.

The mortality pattern is shown in the graph below. The controls woke up 30-90 min after ischemia and suffered periods of intense seizures, dying 48 hrs later. The CHA gerbils woke up 15-24 hrs after ischemia. Initially sluggish, they regained normal behavior within the next 24 hrs. There were no seizure episodes. 7 days after ischemia there were 63% CHA treated survivors but only 52% controls (P<0.001).

Our study shows that even a single posts ischemic injection of CHA considerably improves survival of an otherwise lethal ischemic episode. Further studies on the mechanism of posts ischemic protection by CHA are presently made in our laboratory.

This work was supported in part by the Office of Naval Research grant nr. N00014-9-0471.

417.6 CEREBRAL ISCHEMIA IN GERBILS: REDUCTION IN MORTALITY THAT PRESENTS A MECHANISM WHICH TRANSMITTER RELEASE IS MORE SENSITIVE TO HYPOXIA THAN POSTSYNAPTIC RECEPTORS R.C. Silvia, J.S. Faye, P. Lipton*. (Spon: J.L. Dretchen) Dept. of Physiology, The Upjohn Company, Kalamazoo, MI 49007.

Polymeric forms of prostaglandin B2 (PGB) may stabilise oxidative phosphorylation, act as a potent calcium ionophor (Devlin), and protect the functional integrity of mitochondria subjected to anoxic insult. Since the trimer of PGB-trimer appears to have the greatest effect on mitochondria, we have studied the effect of treatment with PGB-trimer on survival following cerebral ischemia in the gerbil. 150 male gerbils (70-80 g), were anesthetized with 0.5% halothane and 33% N2O, subjected to 15 or 20 min of bilateral cerebral ischemia by occluding both carotid arteries by a suture loop which was verified by direct inspection. Rectal temperature was maintained at 36.5-37.5°C by a heating blanket. Blood pressure, pulse, and O2 saturation were continuously monitored during ischemia. Gerbils received either PGB-trimer dissolved in 4% NaHCO3, 4% NaHCO3 alone, or 0.9% NaCl intraperitoneally. Animals were injected at 30, 60, 90, and 120 min after cessation of ischemia.

Results: There were no differences in temperature, BP, pulse or O2 saturation in the various treatment groups. The results demonstrate significant improvement in survival in animals treated with PGB-tn as compared to either control group following 15 min (P<0.04) or 20 min (P<0.002) cerebral ischemia.

The mechanism of this effect on survival is unknown, but may be the result of PGB effects or calcium flux or protective effects on mitochondrial function. Other possible protective mechanisms include effects in free radical formation and cerebral blood flow.

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It is well documented that pre-ischemic serum hyperglycemia exacerbates ischemic brain injury. However, little data are available on ischemic neuronal cell death in hyperglycemic animals with normal ischemic periods of recovery. The present study evaluated the effect of pre-ischemic serum hyperglycemia on delayed neuronal cell death after one to seven days post-ischemic survival.

Normally fed or fasted male Wistar rats under halothane/nitrous oxide anesthesia were subjected to 10 minutes of forebrain ischemia via bilateral carotid clamping and hemorrhage hypotension. Two separate weight groups (250-280 g or 310-350 g) of non-fasted animals and one fasted group (270-350 g) were evaluated. EEG and evoked potentials were recorded prior to and during ischemia, 1 hour post-ischemia and 1 to 7 days post-ischemia. Morphological analysis was performed on animals sacrificed at 1 to 7 days of post-ischemic survival.

Fasted animals all survived 7 days and had complete qualitative recovery of electrical activity but extensive neuronal loss in the CA1 sector of the rostral third of the dorsal hippocampus. One group of fed rats (250-280 g) all survived 5 to 7 days, but demonstrated persistent deficits in electrical function. In addition to CA1 hippocampal cell loss, scattered cell death was found in other hippocampal areas. However, neuronal loss in numerous cortical regions was also seen. In contrast, post-ischemic survival of fast fed rats was limited to 24-48 hours at which time the animals died or were electively sacrificed due to impending death. Extensive neuronal loss was already present even after 24 hours in these animals and at times involved the entire hippocampus. Extensive cortical and subcortical regions demonstrated both neuronal and glial cell loss. Cell loss was often asymmetric.

These studies suggest that hyperglycemic-induced acidosis potentiates cell death and loss and may be an important factor in extending the pattern of selective vulnerability to brain regions other than the hippocampus in the rat. A possible mechanism for these findings may be that acidosis impairs intramural calcium buffering and enhances post-ischemic neuronal calcium toxicity secondary to abnormal agonist-receptor interactions.

(Supported by NS 19550 and VA-VA Affiliate.)


Delay neuronal death following diffuse forebrain ischemia in rats is well documented. However, neuronal loss data are available on ischemic neuronal cell death in hyperglycemic animals with normal ischemic periods of recovery. The present study evaluated the effect of pre-ischemic serum hyperglycemia on delayed neuronal cell death after one to seven days post-ischemic survival.

Normally fasted or fed male Wistar rats under halothane/nitrous oxide anesthesia were subjected to 10 minutes of forebrain ischemia via bilateral carotid clamping and hemorrhagic hypotension. Two separate weight groups (250-280 g or 310-350 g) of non-fasted animals and one fasted group (270-350 g) were evaluated. EEG and evoked potentials were recorded prior to and during ischemia, 1 hour post-ischemia and 1 to 7 days post-ischemia. Morphological analysis was performed on animals sacrificed at 1 to 7 days of post-ischemic survival.

Fasted animals all survived 7 days and had complete qualitative recovery of electrical activity but extensive neuronal loss in the CA1 sector of the rostral third of the dorsal hippocampus. One group of fed rats (250-280 g) all survived 5 to 7 days, but demonstrated persistent deficits in electrical function. In addition to CA1 hippocampal cell loss, scattered cell death was found in other hippocampal areas. However, neuronal loss in numerous cortical regions was also seen. In contrast, post-ischemic survival of fast fed rats was limited to 24-48 hours at which time the animals died or were electively sacrificed due to impending death. Extensive neuronal loss was already present even after 24 hours in these animals and at times involved the entire hippocampus. Extensive cortical and subcortical regions demonstrated both neuronal and glial cell loss. Cell loss was often asymmetric.

These studies suggest that hyperglycemic-induced acidosis potentiates cell death and loss and may be an important factor in extending the pattern of selective vulnerability to brain regions other than the hippocampus in the rat. A possible mechanism for these findings may be that acidosis impairs intramural calcium buffering and enhances post-ischemic neuronal calcium toxicity secondary to abnormal agonist-receptor interactions.

(Supported by NS 19550 and VA-VA Affiliate.)

417.9 RAINBOW KA DOSE-RESPONSE CURVE: CHANGES IN RAT MOTOR AND NEUROPHYSIOLOGIC PATTERN. E. D. Ruth and J. A. Seimen*. Inst. Study Dev. Environ, and Committee on Neurosci, Univ. Ill., Chicago 60680.

Systemic K[A] injections induce limbic motor seizures and ultimately, widespread limbic brain damage. Using convulsive doses of K[A] alone (1X dose), we previously observed that few neurons are irreversibly injured prior to the onset of convulsively defined status epilepticus (SE). However, within 5 minutes of SE, neurons in the hippocampal CA1 and CA3 sectors are irreversibly damaged. The present study examined the relationship between neural activity and microvascular constriction.

Intraperitoneal K[A] or K[A] (1X dose) was administered to male Sprague-Dawley rats (250-290 g) under pentobarbital anesthesia. EEG and extracellular field potentials were recorded from the granule layer of the dentate gyrus during the first 20 minutes of SE. At the 1X-dose SE onset was preceded by staring, wet dog shakes and automatisms and brief episodes of rearing/forebrain clonus; SE consisted of repetitive, non-tonic neuronal autotomies punctuated by frequent and severe rearing episodes. At the 3X-dose wet dog shakes were completely suppressed, the frequency of rearing declined, and a new behavior emerged characterized by erratic, uncoordinated movements. At the 6X-dose SE behavior no longer appeared, being replaced by pontine tone and ophthalmic component.

Preliminarily, a histopathologic and morphological analysis of brain damage in these cases yielded somewhat unexpected results. As shown previously, within 30 minutes of SE, neuronal damage was present in the hippocampal formation from the 1X-dose. At the 3X-dose there was near-total loss of CA3 pyramidal cells occupying the middle 1/3 of the hippocampus and a patchy CA3 damage in the septal 1/3 of the area. The CA3 damage was limited to the hippocampal CA1 and corresponding parts of the hilar CA3. Curiously, the CA1 hippocampal sector was spared at all dosages, and the remaining hippocampal fields also appeared to be intact. In contrast, destruction within and surrounding the angular/gyrus region was more severe at the 1X-dose, to light at the 2X-dose, to moderate at the 3X-dose and to light at the 6X-dose. The results suggest that (i) the 6X-dose induces a profound and selective cortical lesion; (ii) the temporal 1/3 of the hippocampal formation is highly resistant to the actions of K[A]; and (iii) K[A]-induced damage to the hippocampus may suppress subsequent angular damage and vice-versa.

The intraparenchymal vascular network supplying the brain is paracellular fluid pathways that communicate with the subarachnoid space (SAS). These pathways include the perivascular spaces (PSVS) around arterioles, the basal laminae (BL) around capillaries, and the pia-arachnoid layers. The PSVS can be identified as follows: (a) through paravascular fluid circulation in the CNS and may have significance for studies of the development and/or clearance of cerebral edema. (Supported in part by NIH grant no. 2PSO NS16332-04, 04, NINCDS, NH.)

417.13 AGE, GENDER AND BODY POSITION AFFECT CAROTID ARTERY BLOOD FLOW VELOCITY IN RESPONSE TO VALSALVA STRAIN IN HUMANS. B. L. Cintrich*, B. L. Metger**, and B.A. Thurtell. The University of Michigan, Ann Arbor, MI 48109.

Marked shifts in cerebrovascular blood flow velocity and pressure in response to straining (Valsalva Maneuver) are life-threatening in individuals with cerebrovascular disease (CVD). It is known that the incidence of CVD increases with age, and there is some evidence that gender differences influence the specific form of CVD pathology. In contrast, little is known about reducing or preventing marked hemodynamic shifts. In this study we examined the effects of age, gender and one potential modifier (body positioning) on carotid artery blood flow velocity (CABFV) during straining.

Subjects were placed in a pressure gauge meter to 40 mmHg for 10 seconds. CABFV was measured by noninvasive Doppler technique continuously over phases of the Valsalva Maneuver (baseline, strain, overshoot). 80 younger males and 65 younger females (30-55 yrs.) were tested in 5 positions (flat, side, 30°, and 70° up, and in a sitting position) and 47 older females (over 55 yrs.) were tested in the flat and 70° upright positions. The changes in CABFV were significantly greater in the older females (X = -42% vs -49% respectively; p<.001) and following release (overshoot) of strain (F = 13.17; p<.001) and following release (overshoot) of strain (F = 13.17; p<.001). At overshoot, greater increases in CABFV were observed in younger females compared to older up to 10%.

At 30° and 70° up positions, CABFV was seen in the more reclining as compared to upright positions (X = 65% vs 52% respectively; p<.05). At overshoot, greater increases in CABFV were observed in younger females compared to older up to 10%.

Both age and gender significantly affected CABFV in response to straining. Changes in CABFV were significantly more intense in older as compared to younger subjects. Specifically, older males had greater reductions in CABFV during the period of straining while lying flat than did older females (X = 76% vs 68% respectively; p<.05). At overshoot, greater increases in CABFV were observed in younger females compared to older (X = 29% vs 28% respectively; p<.05).

We conclude that CABFV responses to straining become significantly more intense with age and that gender differences exist. Furthermore, we suggest that the intensity of CABFV changes is modified by body position and can be lessened by more upright positions. Supported by NIH grant # 2 R01 NS01142-03.


Calcium may play a pivotal role in post-ischemic tissue injury. One of the many calcium-activated biochemical processes which may lead to tissue damage is through calcium-activated neutral proteinase (CANP). In a focal ischemic stroke model in the rat (S. T. Chen et al, Stroke 17:738, 1986), we have noted that the calcium accumulation in cerebral cortex is correlated with the lesion; reaction product was limited to surrounding areas of the vascular and white matter of the intact hemisphere. By contrast, the vascular network and white matter of the intact hemisphere were less labelled. These findings support our hypothesis of an ongoing paracellular fluid circulation in the CNS and may have significance for studies of the development and/or clearance of cerebral edema. (Supported in part by NIH grant no. 2PSO NS16332-04, 04, NINCDS, NH.)

We have investigated the effects of the specific PAF [1-alkyl-2-acetylglycerylphosphocholine] antagonist, BN52021, on the accumulation of free fatty acids (FFA) and diacylglycerols (DG) and on the loss of fatty acids from polyunsaturated lipids (polyPLs) in the brain of the Mongolian gerbil following ischemia. The gerbil was decapitated, and brain tissue was collected immediately after ischemia. The brain tissue was homogenized and extracted with chloroform-methanol. The FFA and DG were isolated by thin-layer chromatography. The fatty acid content of the FFA was determined by gas chromatography.

The results showed that BN52021 significantly reduced the accumulation of FFA and DG in the brain. The reduction was more pronounced in the ischemic conditions. The reduction was accompanied by a decrease in the accumulation of polyPLs. Pretreatment with BN52021 decreased the accumulation of fatty acids in the brain, thereby indicating a potential protective effect of BN52021 on the ischemic brain.

417.16 OXYGEN FREE RADICALS AND XANTHINE OXIDASE IN CEREBRAL ISCHEMIC INJURY IN THE RAT. J.S. Beckman1*, T.H. Liu2*, E.L. Hogan2, B.A. Freeman1* and C.Y. Hsu2. Depts. of Anesthesiology and Biochemistry, University of Alabama at Birmingham, Birmingham AL 35233 and Dept. of Neurology, Medical College of South Carolina, Charleston, SC 29425.

The protective effects of the oxygen free radical scavengers, polyethylene glycol-conjugated superoxide dismutase (PEG-SOD); a specific xanthine oxidase inhibitor, allopurinol (AP); and a combination, were investigated in a rat stroke model. Ligation of the right middle cerebral artery (MCA) with 10-0 suture at the level of the rhinal fissure and temporary clamping of both carotid arteries for 90 min resulted in a local infarct of 188 ± 28 mm³ (mean ± SEM) restricted to the right cortex. Ninety min of carotid clamping gives a sub-maximal but highly reproducible infarction volume. In a randomized, blinded study, the volume of cortical infarction was reduced by 30% (34 ± 21 mm³) in rats pretreated IV with 300 μg of PEG-SOD plus PEG-catalase whereas infarct volume in rats pretreated with inactivated PEG-SOD plus PEG-catalase was 187 ± 13 mm³ (p<0.01; n=18). The area of infarction in the right cortex was less than previous results as historical controls, indicating that the protection obtained with active PEG-SOD plus PEG-catalase resulted from scavenging of superoxide and H₂O₂ rather than to catalase activity.

In conclusion, either active or inactive PEG-SOD plus PEG-catalase had no effect on heart rate, systemic arterial blood pressure, or body temperature. A single injection of either active or inactive PEG-SOD plus PEG-catalase was effective up to 24 h post treatment.


Our previous serial intravascular impregnation demonstrated a discrete pattern of neuronal degeneration after brief forebrain ischemia in the gerbil. However, the site of injury in the gerbil was not clear. The finding was that terminal-like silver granules could be demonstrated in the outer two-thirds of the dentate molecular layer (the perforant path terminal zone) 1-3 d after a bilateral carotid occlusion, even though neither the cell bodies of origin of the perforant path nor the dentate granule cells were damaged. To further investigate the identity of these degenerating structures, we performed electron microscopic studies of the dentate gyms in gerbils subjected to transient ischemia. The animals were anesthetized with 2.5% halothane and the carotid arteries were occluded bilaterally for 5 min. The animals were sacrificed either 24 or 48 h later and the hippocampi were processed for electron microscopy. The dentate granule cells were observed at the electron microscope level and the results confirmed the previous findings.

The results showed that the dentate granule cells were significantly damaged after 5 min of ischemia. The electron microscopic studies confirmed the previous findings and provided further insights into the mechanism of injury in the gerbil dentate gyrus. The findings suggest that the degeneration is caused by a specifically targeted mechanism that is different from the mechanism involved in other models of ischemic injury. Further studies are needed to elucidate the specific cellular mechanisms responsible for the damage observed in the gerbil dentate gyrus.
417.19 THE EFFECTS OF ISCHEMIA ON PROTEIN PHOSPHORYLATION IN RABBIT SPINAL CORD. A. Kochhar*, A. J. Zivin, and T. Saltos. Dept. of Neurosciences, School of Medicine, Univ. of California, La Jolla, CA 92033.

Protein phosphorylation systems regulate many diverse intracellular functions in nervous tissue. The physiological activity of substrate proteins depends on their state of phosphorylation, which is determined by the relative activities of protein kinases (which phosphorylate substrate proteins) and phosphoprotein phosphatases (which dephosphorylate phosphoprotein substrates). We examined the effects of ischemia on protein phosphorylation in rabbit spinal cord. Spinal cords were rapidly excised and frozen from control animals or after 1 hr of ischemia which produces irreversible neurologic damage. Tissue samples were homogenized and centrifuged to separate the membrane rich and cytosolic fractions. In endogenous protein phosphorylation assays, membrane and cytosolic fractions were incubated under phosphorylating conditions, in the presence or absence of appropriate protein kinase activators. Proteins were separated by SDS-polyacrylamide gel electrophoresis, stained with Coomassie Blue, and phosphorilated proteins were detected by autoradiography. One hr of ischemia did not affect endogenous phosphorylation mediated by the cyclic AMP-dependent protein kinase system. However, the phosphorylation pattern of both calcium-dependent systems, Ca$^{2+}$-calmodulin-dependent protein kinase and Ca$^{2+}$-phospholipid-dependent protein kinase was altered in the ischemic animals. A 31 kDa phosphoprotein substrate was absent in ischemic cord and phosphorylation of three other substrates (21 kDa, 45 kDa, and 87 kDa) was markedly reduced. These studies show that ischemia selectively inhibits calcium-dependent protein kinase activators. Protocols were developed to study the effects of ischemia on the phosphorylation of a 31 kDa substrate using appropriate protein kinase activators in vitro. There was a marked inhibition of phosphorylation of the substrate in vivo which was reversed by reperfusion. These studies suggest that ischemia selectively inhibits calcium-dependent protein kinase systems. Alterations of these crucial regulatory systems may be responsible at least in part, for irreversible cell death in ischemia.


The ability of a novel and extremely potent inhibitor of iron-dependent CNS tissue lipid peroxidation, U74006F (21-[(2,6-dioxo-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-16a-methylpregna-1,4,9(11)-triene-3,20-dione, monomethane sulfonate), to enhance the early neurological recovery and survival of mice following head injury was investigated. Unanesthetized male CF-1 mice were subjected to a 900 g-cm head injury produced by a 20 g weight dropped 18 cm. This concussive injury resulted in immediate unconsciousness (loss of righting reflex) in all animals and death in approximately 30%. Survivors received a tail vein injection of either vehicle or U74006F (0.001-30 mg/kg) within 5 minutes post-injury. Their neurological status was evaluated 1 hour later using a grip strength test. Administration of a single i.v. dose of U74006F resulted in a significant improvement in the 1 hour post-injury neurological status (grip test score) over a broad dose range (0.003-30 mg/kg) and by as much as 168% at 1 mg/kg. A 1 mg/kg i.v. dose given within 5 minutes and again at 1 hour after a severe injury, in addition to improving early recovery, also increased the 1 week survival to 78.4% compared to 27.3% in vehicle-treated mice (p<0.02). The compound was also effective in enhancing early recovery after a more moderate injury. This study demonstrates that early treatment after severe concussive head injury with a potent inhibitor of iron-dependent lipid peroxidation may significantly benefit the injured brain such that both early neurological recovery and long-term survival are promoted.

418.2 THE 21-AMINOSTEROID, U-74006F, ENHANCES RECOVERY IN A MODEL OF SPINAL CORD COMPRESSION INJURY. D.K. Anderson(a), J.M. Braughler(b), E.D. Hall(b), T.R. Polaceda(a), P.D. Weege(a), and D.K. Anderson(a). (a) Cincinnati VA Medical Center, Cincinnati, OH 45220; (b) CNS Diseases Research, The Upjohn Co., Kalamazoo, MI 49001.

This study was designed to assess the dose-response characteristics and effectiveness of U-74006F to enhance functional recovery in cats subjected to compression trauma of the upper lumbar (L2) spinal cord. The compound is one of a series of novel 21-amino steroids that lack glucocorticoid receptor-mediated activity and are potent inhibitors of lipid peroxidation. At 30 min following injury, treatment was initiated with either vehicle (sterile water) or one of 8 i.v. doses of U-74006F. Initial doses of U-74006F ranged from 0.01 mg/kg to 30 mg/kg. Subsequent dosing consisted of additional i.v. bolus injections followed by a continuous 42h i.v. infusion. Over the 48th treatment period received a total U-74006F dose of 0.048 mg/kg to 160 mg/kg. Treatments were randomized and investigator blinded. Animals were evaluated weekly for neurologic recovery based upon an 11 point behavioral scale. At 4 wk after injury, the spinal cords were removed for histological assessment. With the exception of one dose (16 mg/kg/48h), total doses of 1.6 to 160.0 mg/kg resulted in nearly 75% return of normal neurological function by 4 wk after injury. Lower doses of 0.16 and 0.48 mg/kg/48h were associated with approximately 50% return of normal neurological function which was not significantly better than vehicle-treated controls. The lowest dose tested (0.048 mg/kg/48h) was indistinguishable from vehicle-treated cats that recovered only 20% of their preinjury neurological function by 4 wk. Transverse sections of the lumbar and spinal cord stained with luxol fast blue for myelin and evaluated for cavity dimensions. The section of cord with the largest cavity was digitized using a Videoplan (Zeiss) Image analysis device and programmed calculation. The outside and junction between myelin distribution. The cavity dimension was 49% for controls and 27% for the treatment groups. These results demonstrate that over a 100-fold range of doses, U-74006F is remarkably effective in promoting function recovery in cats subjected to compression trauma of the spinal cord.
418.3 DEVELOPMENT OF AXONAL PATHOLOGIES AFTER GRADED SPINAL CORD CONTUSIVE INJURY IN THE RAT. J. R. Wrathall, L. Fineu* and R. M. Koldun*. Department of Anatomy and Cell Biology, Georgetown University, Washington, D.C. 20007.

We have previously reported quantitative light microscopic studies on tissue loss after graded spinal cord contusive injury (J. Neurosci. Abstr. 11:168, 1985). In order to examine early pathological changes more closely, particularly the development of axonal pathologies, we have now applied antiformaldehyde-stained tissue to the light and electron microscopic level and used stereotactical techniques to initiate a quantitative description of the early phase of tissue loss after graded spinal cord contusive injuries that produce mild or severe chronic functional deficit as measured by the motor score, inclined plane and additional tests of reflex and more complex hindlimb function. Results from all tests were used to generate a combined behavioral score (CBS) for morphometric analysis (Exp. Neurol. 88:108, 1985). At 15 min, 2, 4, 24 and 48 hr after injury, tissue was fixed by perfusion with 2% glutaraldehyde, 2% paraformaldehyde and 2mM CaCl2 in 0.1 M cacodylate buffer (pH 7.3) and 200 μm cross-sections of cord at the epicenter (area of maximal damage) were osmicated with 1% osmium-standardized contusive injuries that produce mild or severe chronic functional deficits seen after spinal cord injury. Naloxone and thyrotrpin releasing hormone in rats have shown promise in mitigating the magnitude of spinal cord injury studies in which cats were used as experimental subjects (J. Neurosurp. 85:209, 1981; Horm. Metab. Res. 30:1: Pettegrew et al., 1985). The present study was designed to examine the effects of naloxone and TRH administration on the functional deficit, as assessed behaviorally, in adult rats following a standardized, reproducible, moderate spinal cord contusive injury.

Adult Sprague-Dawley rats were anesthetized and subjected to contusive injuries that produce mild or severe chronic functional deficit as measured by the motor score, inclined plane and additional tests of reflex and more complex hindlimb function. Results from all tests were used to generate a combined behavioral score (CBS) for morphometric analysis (Exp. Neurol. 88:108, 1985). Rats were then connected to a system that permitted freedom of movement, to variable rate infusion pumps for the allotted times. Naloxone treated rats (n = 10) were administered a 4 mg/kg bolus at 30 minutes post-injury followed by, at 2 mg/kg/hr i.v. infusion of TRH for 4 hours (a dose previously found to be effective in cats, n = 10) or a 20 mg/kg i.v. bolus followed immediately by a 2 mg/kg/hr i.v. infusion of TRH for 4 hours and control rats (n = 9) were administered saline in the same amount and manner. All rats were tested on the inclined plane at 1, 7, 14, 21 and 28 days post-injury by a battery of behavioral tests designed to assess both motor and sensory function (Fine et al., 1983). The rats were killed at 28 days post-injury following either naloxone or TRH administration. Possible explanations for lack of effect include differences in species, vertebral level of injury or relative severity and type of injury. (Supported by NIH-NINCDS, NOI: NS-2-2310).

418.4 LACK OF EFFECT OF NALOXONE OR TRH ON FUNCTIONAL DEFICIT IN RATS WITH CONTUSIVE SPINAL CORD INJURIES. D.W. Howler, F. Fan*, R. Eischkowitz* and J.R. Wrathall. Department of Anatomy and Cell Biology, Georgetown University, Washington, D.C. 20007.

Presently, there is an ongoing search for a drug or treatment therapy which will aid in the alleviation of the motor and sensory deficits seen after spinal cord injury. Naloxone and thyrotropin releasing hormone have shown promise in mitigating the magnitude of spinal cord injury studies in which cats were used as experimental subjects (J. Neurosurp. 85:209, 1981; Horm. Metab. Res. 30:1: Pettegrew et al., 1985). The present study was designed to examine the effects of naloxone and TRH administration on the functional deficit, as assessed behaviorally, in adult rats following a standardized, reproducible, moderate spinal cord contusive injury.

Adult Sprague-Dawley rats (200-225 grams) of both sexes were anesthetized and lines put in place for either s.c. or i.v. administration of drugs. Laminectomies were performed at the TB vertebral levels and standardized, reproducible, contusive injuries produced by dropping a 10 gm weight 5 cm onto the cervical spinal cord at the level of vertebra C7, which resting on the dura of the exposed spinal cord (Exp. Neurol. 88:108, 1985). Rats were then connected to a system that permitted freedom of movement, to variable rate infusion pumps for the allotted times. Naloxone treated rats (n = 10) were administered a 4 mg/kg bolus at 30 minutes post-injury followed by, at 2 mg/kg/hr i.v. infusion of TRH for 4 hours (a dose previously found to be effective in cats, n = 10) or a 20 mg/kg i.v. bolus followed immediately by a 2 mg/kg/hr i.v. infusion of TRH for 4 hours and control rats (n = 9) were administered saline in the same amount and manner. All rats were tested on the inclined plane at 1, 7, 14, 21 and 28 days post-injury by a battery of behavioral tests designed to assess both motor and sensory function (Fine et al., 1983). The rats were killed at 28 days post-injury following either naloxone or TRH administration. Possible explanations for lack of effect include differences in species, vertebral level of injury or relative severity and type of injury. (Supported by NIH-NINCDS, NOI: NS-2-2310).
418.7 EFFECTS OF TRAUMATIC BRAIN INJURY ON REGIONAL CEREBRAL BLOOD FLOW IN RATS MEASURED WITH MULTIPLE INJECTIONS OF RADIOACTIVE MICROSPHERES. J. Yamakami*, T.K. McIntosh and A.J. Faden (SPON: I. Koch). Neurology Service, V. A. Medical Center and Department of Neurology, University of California, San Francisco CA 94143.

The radioactive microsphere method was used to evaluate regional cerebral blood flow (rCBF) change following fluid-percussion (FP) induced traumatic brain injury in rats. Nine rats (450-500 g) were anesthetized with isoflurane (1-0.3%) intubated and artificially ventilated. Cannulae were placed in the left carotid artery via the right subclavian artery and in both femoral arteries. For each injection, 100,000 radiolabeled microspheres (15 µ), Cardiovascular Research Institute, University of California, San Francisco, were injected into both carotid arteries followed by 30-40 µl of saline over the femoral artery at a rate of 0.5 µl/min. In five control uninjured rats, three sequential flow determinations were performed at 1 hour intervals. In four rats, a 2 em craniotomy was made over the left parietal cortex and moderate parasagittal FP brain injury (2.2-2.3 atmospheres) was induced. In the injured rats, three microsphere injections were performed; 15 min prior to injury, 30 min and 2 hours following injury.

In the controls, no significant changes were seen in rCBF, MAP and PCO2 over time. The rCBF (ml/min/g) calculated from the uninjured rats confirmed data reported by others: lower brainstem = 1.16 ± 0.09; left anterior cortex = 1.72 ± 0.19; left posterior cortex = 1.89 ± 0.21; right anterior cortex = 1.49 ± 0.16; right posterior cortex = 1.87 ± 0.23. Following traum, there was a profound and significant decline in rCBF over the area of maximal injury (left posterior cortex); from 2.21 ± 0.28 baseline to 0.53 ± 0.03 at 30 min following injury (p < 0.01), with slight recovery at 2 hours following injury (0.74 ± 0.13, p < 0.05). A small but still significant decrease in rCBF was also observed in adjacent regions ipsilateral to the injury and in the opposite hemisphere as well; lower levels in all areas. These data (1) substantiate the feasibility of multiple radiolabeled microsphere injections to measure rCBF in head injured rats; and (2) demonstrate that there is significant hypoperfusion of the injury region and head injury in rats. It is possible that these rCBF changes may contribute to secondary tissue injury after brain trauma.

Supported by Merit Review Grant from the Veterans Administration to T.K. McIntosh.

418.8 CHANGES IN BRAIN INTRACELLULAR FREE MAGNESIUM INFLUENCE NEUROLOGICAL OUTCOME AFTER EXPERIMENTAL HEAD INJURY. T.K. McIntosh, J. Yamakami, and A.J. Faden. Neurology Service, V. A. Medical Center and Dept. of Neurology, University of California, San Francisco, CA 94143.

Trauma to the central nervous system (CNS) often results in the activation of delayed or secondary injury mechanisms that develop over a period of hours to days following the initial insult. Recent magnetic resonance (MR) spectroscopic investigations have shown that brain intracellular free magnesium [Mg2+] declines in the pathophysiology of fluid-percussion (FP) brain injury proportional to the severity of injury (McIntosh et al., JCBF Suppl., 1987). The present study further examined the role of intracellular free magnesium in the pathophysiology of delayed CNS injury. In Study I, male rats (450 g) were anesthetized (50 mg/kg sodium pentobarbital) and treated at 15 min prior to FP brain injury (2.0 atmospheres [atm]) with either MgSO4 (0.15 mM in 0.25 cc saline, n = 8) (in order to prevent the postinjury fall in intracellular [Mg2+]) or saline (equal volume, n = 8). Saline-treated animals showed a fall in [Mg2+] (from 1.15 ± 0.32 mM, p < 0.05) by 60 min postinjury as measured by 31P MRS. These animals developed a moderate neurological deficit which persisted up to 4 weeks postinjury. In contrast, animals pretreated with MgSO4 showed no significant change in [Mg2+] following FP injury (from 1.07 ± 0.95 mM) as measured by 31P MRS and demonstrated a significant improvement in neurological recovery when compared to saline-treated animals (p < 0.05). This improvement in neurological outcome began at 24 h and continued over the 4 week postinjury study period. In Study II, saline-treated animals were subjected to a 1 hr temporary occlusion of the contralateral CCA. Morphometric analysis showed the occurrence of a consistent loss of CA1 pyramidal neurons in the saline-treated animals which persisted up to 4 weeks postinjury. These results suggest that changes in brain intracellular free magnesium [Mg2+] may contribute to the delayed damage and neurological deficit that follow traumatic brain injury.

Supported by Merit Review Grant #74R from Veterans Administration to T.K. McIntosh.


The systemic administration of monosialoganglioside GM1 ganglioside has been reported to ameliorate outcome following ischemic injury of both mechanical and vari­ous neuronal systems of adult rodent CNS. These GM1 effects, however, in some cases, have been shown to be associated with enhanced neuronal cell survival (i.e. cholinergic, dopaminergic or noradrenergic). Furthermore, GM1 or its inner ester derivative (AGF2) have been shown to have beneficial effects in the early postischemic period following transitory cerebral ischemia in cats, rats or gerbils (for a recent review see G. Tettamanti, R.W. Ledeen, K. Sandhoff, Y. Nomel and G. Toffano: "Gangliosides and Neuronal Plasticity", Liviana Press, Padova, Vol.6).

We now report the effect of AGF2 treatment on the delayed hippocampal neuronal damage occurring following severe transitory incomplete ischemia in adult rats (four- vessel occlusion model; Pulsinelli and Braley, Stroke 10: 267, 1979). In particular, computer-assisted morphometry was used to assess ischemia-induced damage to pyramidal neurons of the CA1 region of the hippocampus. AGF2 was administered to rats only those which exhibited isoelectric EEG throughout the 10 min occlusion period. Saline or AGF2 (5-15 mg/kg) was first injected intravenously one hour following clipping off and continuous monitoring of EEG. Tissue sections one day following injury. Rats were sacrificed 18 hours after last injection. Morphometric analysis showed the occurrence of a consistent loss of CA1 pyramidal neurons in the saline-treated rats, an effect which may be partially prevented by the AGF2 treatment. This effect may perhaps be due to reduction in the degenerative changes which occur in the acute postischemic phase.

418.10 LEVELS OF EDema AND ATPase ACTIVITY AFTER LOCAL CEREBRAL ISCHEMIA: GM1 GANGLIOSIDE REDUCES EDema IN PRIMARY AND PERI-INFARCT AREAS. S.P. Karpiak, P. Hauser*, T.S. Liu, and S.P. Mahadik. Div. Neuro­science, Department of Medicine, Denison University, Gambier, OH 43022.

Our studies of the acute effects of ganglioside injections on CNS injury have shown that GM1 ganglioside treatment reduces edema and associated losses of Na,K-ATPase and intracellular Na+ and K+ [1]. Recently studies show that after global ischemia in the gerbil, monosialoganglioside (GM1 & AGF2) reduces mortality by 50% and protects levels of cortical and hippocampal Na,K-ATPase activity [2]. We have begun focusing our studies on a rat ischemia model which results in a highly localized cortical infarct (C1). The model involves occlusion of the middle cerebral artery (MCAo) followed by permanent ligation of the ipsilateral common carotid artery (CCAo) together with a 1hr temporary occlusion of the contralateral CCA. The MCAo+CCAo results in a localized cortical partial infarct area (C), Other cortical regions (C2, frontal; C3 occipital; C4 temporal) are less affected, representing the peri-infarct zones. At 72 hrs after the MCAo+CCAo we found a greater than 20% decrease in Na+,K+-ATPase activity in region C, with no significant decreases in regions C2-C4. Mg2+ATPase (a marker for mitochondrial function) was decreased by 10% in region C, and slightly increased in regions C2-C4. 72hrs after the MCAo+CCAo we found a 30% increase in water content (edema) in the primary infarct region (C); a 1.3X increase in area C2; a 1.3X increase in area C3; and no change in water content in areas C4. Rats treated (i.p.) with GM1 immediately after the MCAo+CCAo (40mg/kg) and 24hrs later (200mg/kg) showed a 50% reduction in water increases in regions C2,3,4. Acute GM1 treatment significantly reduced levels of edema in the primary infarct area. The elimination of edema by GM1 may have been achieved either by preventing the accumulation of water in the cellular edema or by reducing the extent of ischemia by limiting the peri-infarct zones. Hence, GM1 and AGF2 may be useful in the treatment of acute ischemia by limiting the peri-infarct zones.


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MPP+ increases extracellular potassium in rat striatal slices: Preliminary evidence that consequences of MPP+ neurotoxicity are dopamine-dependent. W.A. Pulsinelli and G.A. Block* (SPON: F. Plum), Cornell Medical College, New York, NY 10021.

MPP+ (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) produces a clinical syndrome similar to idiopathic Parkinson's disease in primates. The neurotoxicity of MPP+ (1-methyl-4-phenylpyridinium) has been shown to be selectively destructive to dopaminergic neurons, although striatal hypermetabolism occurs after ischemia, these alterations do not appear to significantly contribute to striatal nerve cell death.

MPP+ increases extracellular potassium in rat striatal slices: Preliminary evidence that consequences of MPP+ neurotoxicity are dopamine-dependent. W.A. Pulsinelli and G.A. Block* (SPON: F. Plum), Cornell Medical College, New York, NY 10021.

MPP+ increases extracellular potassium in rat striatal slices: Preliminary evidence that consequences of MPP+ neurotoxicity are dopamine-dependent. W.A. Pulsinelli and G.A. Block* (SPON: F. Plum), Cornell Medical College, New York, NY 10021.

MPP+ increases extracellular potassium in rat striatal slices: Preliminary evidence that consequences of MPP+ neurotoxicity are dopamine-dependent. W.A. Pulsinelli and G.A. Block* (SPON: F. Plum), Cornell Medical College, New York, NY 10021.
Guanethidine, an adrenergic neuron blocking agent, produces marked and permanent destruction of the sympathetic nerve system of rats, hamsters and monkeys when administered chronically in high doses. Neuronal destruction appears to occur by a cell-mediated mechanism. It is completely prevented by concurrent treatment with nerve growth factor (NGF), possibly because NGF blocks the release of a substance which induces the immune response. Experiments were conducted to identify novel proteins (potential antigens) induced by guanethidine and their effects on immune responses. Only one-week-old rats and mice were treated daily with 50 mg/kg of guanethidine sulfate for various lengths of time alone or concurrently with 5 mg/kg of NGF. Immunohistochemical studies of control and treated superior cervical ganglia (SCGs) were assessed by 3-dimensional gel after the last injection (i.e., 5 days following the first injection) the animals, along with age-matched controls, were perfused with 5% glutaraldehyde.

Additional studies showed that the atrophy progresses distally and 1 and 3mm from the DRG. Histogram constructed from axonal diameter, area, perimeter and myelin sheath thickness (MST). Shape factor, and indicator of deviation from circularity, was also calculated. SCG nerve branches and muscles in the hindfoot were also examined by light microscopy.

Dorsal root fibers appeared less circular in shape at the DRG and 1 and 3mm from the DRG. Three survival times were given for AC (75mg/kg) followed by 4 daily intratrigeminal injections (300mg/kg) after it follow for last injection (i.e. 5 days following the first injection) the animals, the controls, along with age-matched controls, were perfused with 5% glutaraldehyde.

Electrophysiological recordings from the isolated nervous system of the tadpole reveal phase-locking between bursts of nonspontaneous impulses in the tail and hindlimbs during fictive locomotion, suggesting that the pattern generators that produce coordinated movements of the tail and limbs are not affected by the same spinal circuits. The experiments presented here were conducted to determine whether or not circuits providing a protective role in autoimmune responses.


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Mechanisms of Motor Activity in the Isolated Lumbar Cord of the Chick Embryo Investigated by Ventral Root Current Injection and Inhibitory Blockade. Michael J. O'Donovan and M.A. Berry*. Department of Physiology and Biophysics, University of Iowa, Iowa 52242.

The goal of the present experiments was to establish the influence of motoneuron membrane properties and synaptic inhibition on the pattern of motor activity generated by the isolated spinal cord of 6-8 day (st.26-32) chick embryos. Electrophysiological recording from ventral roots and muscle nerves were used to monitor motoneuron excitatory drive. A new technique - ventral root current injection - was used to polarize the membranes of a population of motoneurons to examine their excitability and the voltage dependence of their synaptic potentials. The use of a bridge circuit enabled current to be injected through the ventral root electrode while simultaneously recording the motoneuron depolarization. The ability of current injected through the ventral roots to polarize motoneuron membranes was verified in older embryos using simultaneous intracellular recording.

The electronic recordings revealed that motoneurons were depolarized during each cycle of activity but discharge was largely restricted to the early part of the depolarization. In the youngest animals (st.26-28) the depolarization was briefer than at other stages. The amplitude of the depolarization was increased by hyperpolarizing current and reduced by depolarizing current injected into the ventral roots. Prolonged membrane hyperpolarization and sudden depolarizing current steps did not block or trigger motoneuron activity. Similar findings were obtained in older embryos using intracellular recording and suggest that the depolarization is the product of chemical synaptic action and not an intrinsic property of motoneurons. Stage 30 motoneurons were also capable of maintained discharge in response to depolarizing current injection, which suggests that the absence of late motoneuron discharge during prolonged synaptic depolarization is not simply due to an immaturity of repetitive firing capability. Path analysis of inhibitory blockers (picrotoxin $10^{-4}$ M; Strychnine $10^{-5}$ M) reduced the rate of spontaneous motor activity and increased both the amplitude of the depolarization and the intensity of motoneuron discharge. The results indicate that motor activity is produced by similar synaptic mechanisms in young and old embryos and that synaptic inhibition is involved in the genesis of motor activity very early in development. Supported by NSF BNS 8402838.

419.5 EMERGENCE OF FLEXION AND EXTENSION MUSCLE SYNERGIES IN THE HINDLIMB OF CHICK EMBRYOS. N.S. Bradley and A. Bekoff. Dept. EPO Biology, University of Colorado, Boulder, CO 80303.

EMG recordings of motor unit activity in intact chick embryos (Bekoff, A., PNAS, 72:1245, 1975) and isolated spinal cord-hindlimb preparations (Landmesser, L.T., and M.J. O'Donovan, J. Physiol, 347:189, 1984) indicate intrinsic motor unit muscle patterns are present at 7 to 10 embryonic days. It has been proposed that the patterns emerge sequentially: 1st, muscles with synergistic or antagonistic action at a joint that are synchronously or reciprocally activated; 2nd, muscles with antagonistic action at a joint that are synchronously or reciprocally activated; 3rd, these patterns are coordinated both by the amplitude of the depolarization and the intensity of motoneuron discharge. The results indicate that motor activity is produced by similar synaptic mechanisms in young and old embryos and that synaptic inhibition is involved in the genesis of motor activity very early in development. Supported by NIH grant NS 20310.

419.6 ORGANIZATION OF VESTIBULO-OCULAR PROJECTIONS IN THE CHICKEN EMBRYO. G. Petursdottir and J.C. Glover. Institute of Physiology, University of Oslo, Oslo 1, Norway.

We are studying the organization and development of the vestibulo-ocular projections of the chicken embryo. Using retrograde tracing techniques, we have demonstrated previously that vestibular spinal neurons can be segregated into 3 discrete, coherent groups that project to the spinal cord by different pathways (Glover et al., 1986, Soc. Neurosci. Abst. 12:1191; 1989). The spatial segregation is apparent at least as early as the time that the vestibulospinal axons first reach the spinal cord. Since the vestibulospinal axons are monoaxonic, they are organized into discrete, coherent groups on the basis of their projection pathway. Orthograde tracing is being attempted to determine how early this organization is expressed.

An in vitro preparation of the brainstem was used for the determination of vestibulospinal neuron projections. The results indicate that the vestibulospinal neuron projections to the interstitial nucleus of Cajal are segregated into discrete, coherent groups on the basis of their projection pathway. The results indicate that the vestibulospinal neuron projections to the interstitial nucleus of Cajal are segregated into discrete, coherent groups on the basis of their projection pathway. Therefore, the vestibulospinal neuron projections to the interstitial nucleus of Cajal are segregated into discrete, coherent groups on the basis of their projection pathway.

A KINETIC STUDY OF CHICK EMBRYONIC MOTILITY. S.J. Watson* and A. Bekoff (SPON: S.M. Royer). Dept. EPO Biology, University of Colorado, Boulder, CO 80303.

Spontaneous motility of the chick embryo in ovo has been described from behavioral observations as consisting of periodic sequences of random and Jerky movements of the trunk, neck and limbs (Hamburger, V., Quart. Rev. Biol., 38:342, 1963). Hamburger reported that there was no coordination between body parts, such as the two legs, and no information was provided on the nature of coordination within a limb. However, EMG analysis of leg muscles has demonstrated a high degree of coordination of activity between leg muscles (Bekoff, A., et al., PNAS, 72:1245, 1975), and coordination between knee and ankle synergistic muscles by 9 days of incubation (Bekoff, A., Brain Res., 162:271, 1976). Hence, EMG data support the view that the neural circuitry underlying intralimb coordination is organized at early stages in development even though the behavior appears random and uncoordinated to the observer. Using cinematographic methods, we sought to obtain a more quantitative description of embryonic leg movements and to determine whether there is a consistent pattern of coordination between limb segments.

A window was made in the shell of fertilized eggs on embryonic day 9 or 10 and the extraembryonic membranes were displaced to reveal the embryo positioned on its left side. The lateral aspects of the sacrum, hip, knee and ankle of the right leg of the embryo were marked with a dot of black paint. Spontaneous movements of the leg were video recorded at 16.7 frames per second and a computer program was used to digitize joint angles frame-by-frame. Cycles of flexion and extension that produced relatively large excursions of the leg were chosen for analysis. For the leg movements analyzed so far, cycle period varied from 170 to 2000 ms. Typically, the leg and ankle joint sequences showed a gradual wobble and flexion-sustained synchrony. Motion at the hip could be synchronously paired with corresponding motions at the distal joints, synchronously paired with the reverse motions at the distal joints, or show very little angle change during the cycle.

These data demonstrate that movement of an individual leg during embryonic motility is not random. Rather, joint motions can be coupled in specific patterns of coordination. Furthermore, these results are not only consistent with studies referenced above but demonstrate that the organized motoneuronal output is reflected in the resulting leg movements. Supported by NIH grant NS 20310.

We have demonstrated previously that fetal spinal cord transplants support neuronal survival and axonal elongation in the injured immature spinal cord. The current experiments were designed to determine whether these transplants also promote recovery of motor function following neonatal spinal cord lesions. A total of 18 pups were used in this study. A spinal cord hemisection was made at a mid-thoracic level in 12 rat pups (24 hrs. old). In half of these animals a transplant of fetal spinal cord tissue (E14) was placed on the lesion site (HXTP) while the others received no transplant (HX). Six littersmates served as controls (CON). Injections following an error or a locomotor step pattern similar to that in CON animals (CON=67±5mm, HX=34±5mm). None of the animals received the transplants demonstrated qualitative and quantitative improvements in several parameters of locomotion. This alteration indicates that neural tissue transplants are able to mediate recovery of motor function after spinal cord hemisection. Supported by: PVA #NBR-5072, NIH NS19259, and MOD #5-448.

POSTNATAL MATURATION OF LATERAL GASTROCNEMIUS MOTOR UNITS. A.W. English and M.K. Cook*. Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA 30322, SPON: R. Fricke

Architectural and morphometric analyses of the rat lateral gastrocnemius (LG) indicate that LG muscle fiber number doubles by the fourth postnatal (P4) day and reaches the adult size by P32 in each of its three unipennate heads. This increase is due to the rapid differentiation of secondary myofibers. In light of evidence that central nervous organization proceeds from the hindlimb to the head, it was hypothesized that the animals to use a locomotor step pattern similar to that in CON animals (CON=67±5mm, HX=34±5mm). None of the animals received the transplants demonstrated qualitative and quantitative improvements in several parameters of locomotion. This alteration indicates that neural tissue transplants are able to mediate recovery of motor function after spinal cord hemisection. Supported by: PVA #NBR-5072, NIH NS19259, and MOD #5-448.

POSTNATAL DEVELOPMENT OF COMPARTMENT NUCLEI OF CAT LATERAL GASTROCNEMIUS. A.W. English and M.K. Cook*, Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA 30322. A compartment nucleus is the collection of motoneurons which innervates a neuromuscular compartment. In adult animals the compartment nuclei are arranged in a topological manner in the spinal cord (Weeks and English, J. Comp. Neurol. 235:255-267, 1985). Reports of motoneuron cell death in the early postnatal period and the suggestion that the neonatal motor neurons might have multiple long branches prompted us to investigate ON arrangement in the spinal cord of kittens. Kittens ranging in age from postnatal day six (P6) to P32 were anesthetized with ketamine HCl. On one side of the animal a single primary branch of the nerve to lateral gastrocnemius and soleus (LG-S) was cut and soaked in a solution of horseradish peroxidase (HRP) for 2 hours. Each primary branch of the LG-S nerve is known to innervate a single neuromuscular compartment in adults. On the other side of the animal, the entire LG-S nerve was treated similarly. After a 48 hour survival period, the animals were sacrificed and the LG-S spinal cord segments sectioned and reacted for demonstration of HRP. The number of labeled LG-S motoneurons in kittens is significantly different from that of adult cats, which indicates that no significant postnatal cell death is found in this motor nucleus. The number of motoneurons labeled after soaking different primary muscle nerve branches is also not significantly different from the number found after soaking the same branch in adult cats. Thus, no extensive pruning of long axon branches to different compartments is found. In all instances a bimodal distribution of motoneuron somatic sizes is found. These alpha and gamma motoneurons in each ON are more different from those of adult cats, which indicates that no significant postnatal cell death is found in this motor nucleus. The number of motoneurons labeled after soaking different primary muscle nerve branches is also not significantly different from the number found after soaking the same branch in adult cats. Thus, no extensive pruning of long axon branches to different compartments is found. In all instances a bimodal distribution of motoneuron somatic sizes is found.
ACTIVITY DEPENDENT REGULATION OF ACETYLCHOLINE RECEPTORS IN POSTNATALLY DEVELOPING NORMAL AND MED MOUSE MUSCLE. Joanne H. Yeakley* and G. Cary Richens. Dept. Of Biology, Pomona College, Claremont, CA 91711

We have investigated whether muscle activity is necessary for the postnatal loss of extrajunctional acetylcholine receptors (AChRs) that normally occurs in rodent muscle. To do this, we have used mice with motor endplate disease (med mice), a hereditary recessive disorder that causes failure of neuromuscular transmission due to decreased action potentials, and thus loss of muscle activity, at about 10d after birth, during the period of postnatal decline of extrajunctional AChR.

We examined AChRs in biceps brachii (long head) and rectus femoris muscles, which are severely affected by denervation, in med mice and in normal littermates between 5 and 31d after birth. In normal mice, AChR levels, determined by [125I]-n-bungarotoxin binding assays, declined from about 40 fmol/mg of tissue wet weight at 5d to about 2 fmol/mg at 21d. In med littermates, AChR levels followed the normal time course until about 10d, then rose to about 20 fmol/mg (biceps) or 12 fmol/mg (rectus) by 21d. We established that at least 80% of the AChR in these muscles is surface, not internal, AChR. In muscles in which milli current-induced muscle inactivity prevents and reverses the normal postnatal decline in extrajunctional AChR levels.

We have also determined the effect of denervation on AChR levels in muscles from normal and med mice. Maximum levels were observed by 6d after denervation in both normal and med rectus, AChR levels in 15-25d med rectus muscle that had been denervated for 4-15d rose from 11.9 to 25.8 fmol/mg in rectus and from 17.7 to 21.0 fmol/mg in biceps. In normal muscles under the same conditions, AChR levels rose to 35-40 fmol/mg. It is possible that the degree to which AChR levels rise after denervation is inversely related to the fraction of muscle fibers previously inactivated by the med disorder. We are currently testing this hypothesis.

The data do not exclude a role for activity-independent factors in the regulation of AChR levels in neonatal muscles, but they clearly show that nerve-induced muscle activity is important in the postnatal loss of extrajunctional AChRs.

Supported by NSF Grant BNS 83-02712 and by the Seaver Science Fund of Pomona College.


The purpose of this study was to examine the postnatal changes in diaphragm (DIA) fatigability and oxidative capacity. Diaphragms were excised from newborn rats killed at 2, 3, 6 and 30 weeks of age. Strips of muscle were cut from the mid-costal region and suspended in a temperature controlled bath containing Krebs' solution. Isometric contractions were evoked by stimulation of the phrenic nerve, and fatigue resistance determined by repetitive stimulation at 40 Hz for 330 msec trains with 1 train presented every 3 min. Locomotion was scored (at 10 sec intervals) by the formula: score = (total number of pedal waves - number of pauses)/(number of locomotion long before it is spontaneously used. Other 1 locomotor programs.

Escape locomotion differed from spontaneous locomotion in two ways: 1) it was much more rapid; and 2) at each stage it appeared to be triggered by a locomotion (and possibly a sensory) system that can be triggered by serotonin and modulated by neuropeptides (Henning et al., 1979; MacKay and Carew, 1980). Since there is such a dramatic difference between juvenile and adult locomotion, it will be of interest to examine the juvenile locomotor circuit as it takes on these adult properties.

Dendritic reorganization of motoneurons has been studied in the invertebrate hawkmoth as the motoneurons are responsive to innervate new myofibers during metamorphosis (Levine and Truman, '85). Similar dendritic changes have not been studied in vertebrates which undergo metamorphosis. This may be significant since these changes could underlie alterations in behavior, such as the transition from filtering to predation in frogs. Metamorphic reorganization of the jaw in Rana pipiens is characterized by turnover of the jaw muscles, embryonic myofibers degenerate and secondary myofibers are born. During this transition, motoneurons are responsive to innervate new target cells, the secondary myofibers. In this respect we describe the changes in dendritic morphology by the trigeminal motoneurons that are responsive during metamorphic changes. Changes in feeding behavior from tadpole to adult are not explained by addition or subtraction of motoneurons, since motoneurons are not added to the trigeminal motor nucleus by neurogenesis or migration, nor are motoneurons deleted from the nucleus by cell death (Alley and Barnes, '83). It would be of interest to determine if reorganization of trigeminal motoneurons are reorganized as are the adult motoneurons of hawkmoths. The branch of the mandibular portion of the trigeminal nerve supplying the posterior lower jaw of R. pipiens is described and schematized in this report.

In adult frogs the dendrites of trigeminal motoneurons ramify in two spatially discrete zones including the ventrolateral and the dorsomedial medulla (Matus and Sefton, '78). In contrast, we found that the dendrites of the motoneurons from premetamorphic tadpoles are oriented almost exclusively in the ventrolateral nuclei, additional, we have observed developmental changes in the somatodendritic geometry in premetamorphic tadpoles including increases from birth to stage XX of primary dendrites, 2) diameter of primary dendrites, 3) length of dendrites, 4) size of the trigeminal nucleus and 5) somal volume. As these motoneurons are recruited during the transition from larva to adult, their dendrites have the potential for a dramatic increase in synaptic connectivity as a mechanism to establish the new feeding behavior present in the adult.

Supported by NIH grant # DE07620.


A substantial ipsilateral corticospinal (CS) projection is likely to issue from the surviving motor cortex of adult rats subjected neonatally to ablation of the opposite motor cortex (Hicks and D'Amato, Exp. Neurol. 92, 375-387, 1986). It has also been shown with modern axonal tracing methods that a small ipsilateral CS projection exists in the neonatal rat (Plesser and Beaudet, J. Neurosci. 11:1284, 1985). In this study, we obtained further information about the cortical origins, course and spinal terminations of the ipsilateral CS projection in the normal adult rat. To trace the pathway anterogradely, ten, 0.1 uL injections of 2% WGA-HRP were inserted into one cut CS tract at the C6-C7 level of the cord, and their cerebral cortices were examined for the presence of retrogradely labeled neurons. As a control for possible diffusion of the tracer to, and injection of the tracer, and their cerebral cortices were examined for the presence of retrogradely labeled neurons.

Our major results were as follows: (1) Ipsilaterally coursing CS fibers were found in ventral and dorsal columns, chiefly in the latter. (2) Unlike the crossed projection, which terminates principally in the dorsal horn (Lanfranco et al., 1991), the CS input terminates chiefly in the spinal intermediate zone (laminas VI). (3) Ipsilaterally projecting CS neurons were found throughout the major zones, with the exception of the nonspinal terminations (both forelimb and nonmotor areas). (4) Ipsilaterally projecting cells resided in the ventral one-third of cortical laminas V and VI, as well as contralaterally projecting cells are distributed throughout lamina V. (5) The ratio of contralaterally to ipsilaterally projecting cells is the order of 4:1 (3:1).

Thus, a small but significant ipsilateral CS projection from areas of the motor cortex (in the intermediate zone of the spinal cord). The enhancement of this projection through axonal sprouting or other processes may underlie in part the much larger and stronger ipsilateral projection seen in the neonatally lesioned animal. (Supported by NIH Grant NS 20146).

419.16 THE TIMING OF SWIMMING IS CONTINGENT ON METAMORPHOSIS FOLLOWING RECOVERY FROM SPINAL TRANSECTION IN THE SEA LAMPREY. L. Margolin* and J. Alves. (SPONSOR: R. Schatz). Dept. of Biology and Marine Science Center, Northeastern University, East Point, Nashua, MA 03080.

Temperature has been shown to have important effects on the quality of recovery from spinal cord transection. Temperature effects on pre-metamorphic specimens parallel the changes observed in period, propagation time, and phase lag that are observed in ammocoetes (Soc. Neurosci. Abstr. 12: 1574). The specimens that recovered at the colder temperature took longer but recovered more normal behavior. In contrast, post-metamorphic specimens exhibit changes in timing that are opposite from those in larvae. There was no difference in amplitude for both pre- and post-metamorphic specimens at the two temperatures (as in ammocoetes).

The above results indicate that metamorphosis has a greater effect on the timing of recovered swimming undulations, than does temperature. This contrasts with the greater effect of temperature on the chronology of recovery influence the development stage (Wibul, et al., this volume). Supported by NSF Grant BNS-8406880.


Previous studies have established that intracortical microstimulation (ICMS) within the surviving motor cortex will evoke bilateral, contralaterally projecting motor responses characteristic of a motor neuron's innervation, termed the enhanced projection (Humphrey and Nation, Soc. Neurosci. Abstr. 11:1284, 1985). In this study, we extended these observations to metamorphosing sea lampreys, to examine the potential for a dramatic increase in synaptic connectivity as a mechanism to establish the new feeding behavior present in the adult.

At 8°C, the enhanced projection seen in neonatally lesioned animals was observed at 300 Hz and 0.2 msec pulse width (300 pps, 0.2 msec pulse width). The enhanced projection was observed in the adult rat, and suggest that its enhancement through axonal sprouting or other mechanisms may contribute substantially to formation of the more powerful ipsilateral system that exists in the neonatally lesioned animal. (Supported by NIH Grant NS 20146).
Joint movements and their regulatory mechanisms in subjects subjected to various behavioural situations. When applied to relaxed muscle, vibrations generally induced MU discharge (1 to 2 sec) and were able to drive every MU discharge without any latency. The contraction times of the MU twitches ranged from 15 to 70 ms, the majority (53%) lying between 25 and 35 ms. The twitch force was about 5 mN to 20 mN. Each MU which was triggered by an intentional muscle force increase (0-10 kg) performed at low velocities (1-2 mm/sec) began to fire at a precise force level. As from 1, the majority of the MUs had short twitch contraction times (15-35 ms), whereas the MUs discharging at lower force levels were characterized by longer twitch contraction times. MU maximal discharge frequencies ranged between 15 and 30 Hz and were characterized by a noticeable variability of spike time intervals.

The contraction times of the MU twitches were: 1. Atrophy, which varies from slight to severe. There is a reduction in muscle bulk. In order to investigate the influence of this chromosomal abnormality on the ultrastructure of skeletal muscle, serial sections were stained with hematoxylin and eosin. 2. Presence of macrophages in the extracellular space. 3. Recovery of locomotion to monopodal reflex thresholds and responses progressively decrease toward more normal values, and a plantigrade stance begins to recover, as does locomotion on the 5cm runway, which requires accurate foot placement. The step cycle pattern obtained from measurements of joint angle excursions for the hip, knee, and ankle is altered during the recovery period. These kinematic changes are associated with the following stages of motor recovery: 1. Recovery of supraspinal control in the execution of postural reflexes, 3. Recovery of locomotion demanding precise movement.

Patients with Turner's syndrome usually have short stature, steeper from lack of muscle strength, and examination may reveal a striking loss of IIB myosin was noted in the EDL. The proportion of fibers containing IIA myosin was seen in DN EDL. By 3 months after DN a further to the slowing. This study was undertaken to compare the effect of denervation on the isoforms of myosin in the soleus (SOL), a fast-twitch muscle of the C57BL/6J mouse after short (1 month) and longer (3 months) periods of denervation. The right hindlimbs of 4-6 month old anesthetized C57BL/6J mice were denervated by surgical removal of a segment of the sciatic nerve in the upper thigh region. Myosin isoforms were detected and visualized immunohistochemically. Monoclonal antibodies directed against the heavy chain of IIB and IIA forms of fast myosin as well as slow myosin (generous gift of Dr. S. Sciaffino, Padua, Italy) were applied to frozen cross-sections of the majority (53%) lying between 25 and 35 ms. The twitch force was about 5 mN to 20 mN. Each MU which was triggered by an intentional muscle force increase (0-10 kg) performed at low velocities (1-2 mm/sec) began to fire at a precise force level. As from 1, the majority of the MUs had short twitch contraction times (15-35 ms), whereas the MUs discharging at lower force levels were characterized by longer twitch contraction times. MU maximal discharge frequencies ranged between 15 and 30 Hz and were characterized by a noticeable variability of spike time intervals.

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Amphetamine administration enhances the rate of recovery of beam-walking in rats after corticectomy lesions. We have been studying the suitability of beam-walking in rats as a model for predicting the effect of drugs on recovery of function after stroke. We recently demonstrated that the number, but not the timing, of trials on the beam is critical to amphetamine-facilitated recovery. "Prodding" the animals alone promoted recovery but did not enhance the amphetamine effect.

In the present experiments, the effects of trial interval, prodding and pre-training on beam walking scores were studied. All ratings were performed by blind observers using a seven point scale with excellent inter-observer agreement (Kappa > .93).

Sham-operated control rats showed a significant fall-off in performance when six trials were given sequentially over 12 minutes. This decrement could be eliminated by "prodding" and reduced by pre-training or longer trial intervals. Confirming our previous work, lesioned animals tested sequentially showed an increased rate of recovery when subjected to "prodding" alone. The improvement persisted 24 hours after the last sequential trial.

These results indicate that: (1) sham-operated controls normally show a decrement in beam-walking performance when subjected to frequently repeated trials, (2) the fall-off in performance could be eliminated by "prodding" control animals and, (3) "prodding" alone improved recovery in lesioned animals. These data in conjunction with our previous experiments show that the way in which beam-walking is performed can influence the effect of pharmacological agents on recovery of function.

(Supported by NS 06233, NS 07274, and the V.A.)

**DISTRIBUTION OF SCORES (0-10) FOR FOUR-POINT MOTOR ABILITY IN MTS CHRONICALLY INJURED AT DIFFERENT POSTNATAL AGES***

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<th>SCORE</th>
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The cord was totally transected at 0 (4 hrs), 7, 14, 21 and 28 days after birth. All rats were equally divided groups. The pressure applied in group A, B, and C was approximately 20 Kg/cm2 for 5 minutes, 10 Kg/cm2 for 20 minutes and 10 Kg/cm2 for 2 minutes respectively. The nerve conduction measurement on the caudal nerve was taken before and after compression. The nerve was stimulated both proximally and distally to the site of compression, and the compound muscle action potential (CMAP) was recorded with a surface electrode from the segmental muscles of the tail. The motor conduction velocity (NCV) of the compression segment, and the ratio of the proximal/distal CMAP-amplitude (p/d AMP) were calculated to evaluate the degree of demyelination and conduction block of the compressed nerve respectively. The period of initial complete block was significantly longer in group A (9.5±4.6 days) than in group B or C, but no significant difference occurred between groups B and C (both 2.4±0.5 days). Once the conduction block began to recover, the recovery rate of conduction block (recovery rate of P/d AMP) was not significantly different among the three groups. The recovery rate of demyelinating lesion (recovery rate of NCV) was not significantly different among the three groups either. Complete recovery of both NCV and P/d AMP was noted after 37.5±5.9 days in group A, 31.2±4.2 days in group B and 29.4±3.0 days in group C. This study provides an animal model for investigating therapeutic effects of various interventions on neuropathy.
**420.1 BIOCHEMICAL AND MORPHOLOGICAL PROPERTIES OF MICROGLIA IN CULTURES OF RAT CEREBRAL CORTEX CELLS. W.B. Thomas, D.L. Pratt, R.J. Nycander and F.L. Jordan. Department of Physiology and Anatomy, Meharry Medical College, Nashville, TN 37228.**

While microglial cells are considered intrinsic macrophages and thought to be involved in the immune response in brain tissue, many aspects of their cellular function are unclear. These cells have not been highly investigated in CNS cultures containing mixed populations of cells. Microglia were investigated here in tissue cultures of dissociated cerebral cortex from embryonic rat. Three different forms of microglial cells were identified in relatively mature cultures on the basis of morphological criteria, esterase histochemistry, and immunohistochemical staining for Fc receptors and macrophage-associated antigens. Two of these forms corresponded to the amoeboid and ramified microglia described in vivo; the third, unique morphological form possessed moderate non-specific esterase activity and stained comparably for Fc receptors and macrophage-specific components. Using latex beads (1.5 µm diameter) to assess phagocytic activity, only the amoeboid and filamentous-bearing forms exhibited this capability. The filament-bearing form was the predominant type of microglia observed in the cultures; however, the exact nature of its relationship to the amoeboid or ramified cells is uncertain. It cannot be distinguished at present whether this form corresponds to the "reactive" microglia described in vivo which is thought to be derived from the ramified cell type. An intersubtype relationship between the amoeboid and ramified cells was not observed. Moreover, the identification of these cells supports the potential of this culture system to investigate functional aspects of microglia and their interaction with brain cells.

(Supported by NIH-MCB 860037, NSF R11-831859 and ORN 00014-86-K-0489)

**420.2 DETERMINATION OF THE SOMATOSTATIN-CONTAINING NEURONAL POPULATION IN PRIMARY CULTURES OF CEREBRAL CORTEX. E.L. Jordan and W.R. Thomas. Department of Physiology, Meharry Medical College, Nashville, TN 37228.**

Somatostatin was initially identified in the hypothalamus as an inhibitory factor for the release of growth hormone, and subsequently shown to arrest the release of biologically active substances in several other tissues. Extra-hypothalamic regions of the brain also contain somatostatin. Based on the evidence somatostatin has been suggested to function as a transmitter of neurons intrinsic to the cerebral cortex; however, the synecptic physiology of somatostatineric neurotransmission has not been characterized. As an initial step in the characterization of the physiological role of somatostatinergic neurons, the antigenic expression of this peptide was examined in primary cultures of rat cerebral cortical cells. In this preparation, the exact number of somatostatin-containing neurons could be determined, and a complete morphological assessment of this neurochemical cell group performed.

Somatostatin-like immunoreactivity was detected using an indirect immunofluorescent staining technique. Specific immunoreactivity appeared to be localized only in neurons. Staining activity began to appear in some neurons in the cultures during the second week in vitro and increased in intensity over the third week. The proportion of this somatostatin-containing neuronal subpopulation was determined by direct cell counting. A percentage of 1.25 of the total neuronal somatostatin based on multiple counts from six different cell platelets. Somatostatin somatostatin-containing neurons corresponded to the four major morphologies of pyramidal, stellate, bipolar and bifurcated; detected; however, the majority of stained neurons exhibited bipolar (61%) or stellate (35%) morphology. While these bipolar and stellate cells varied slightly in their detailed morphology, they were consistently small in size. All were less than 15 µm in somal diameter, and most were less than 15 µm. These findings define the number and nature of putative somatostatinergic neurons in this cortical culture system. The identification of these cells supports the potential of this culture system to investigate functional aspects of microglia and their interaction with brain cells.

Recently, heterogeneity of proopiomelanocortin (POMC) mRNA content in pituitary anterior lobe corticotrophs and intermediate lobe melanotrophs has been demonstrated by in situ hybridization of tissue sections (Chronwall, et al., Endocrinology. 120:1201, 1987). We have extended for heterogeneity of POMC mRNA content in acutely dispersed rat corticotrophs and melanotrophs. Trypsin dispersed cells were allowed to settle and were exposed to poly-L-lysine coated coverslips prior to paraformaldehyde fixation and subsequent RNA isolation. RNA was labeled with 35S-labeled POMC or 125I-rhodamine. Studies of 125I-labeled cell content were undertaken to insure that the apparent cellular POMC heterogeneity is not inherent to in situ hybridization. 125I-labeled mRNA has been found at comparable levels in all tissue sections and is thought to be a "house keeping" gene expressed at similar levels in all cells (Miller and Shelfes, Nucl. Acids Res., 11:547, 1983). Such mRNA would be expected to have a homogenous distribution relative to POMC from its small size. Tissue sections were used to control for methodologic sources of grain density heterogeneity. Five % of the cells in the anterior pituitary lobe cell suspension and 9% in the cells in the intermediate lobe cell suspension were positive for POMC mRNA. Excessive heterogeneity of silver grain densities was seen over POMC containing cells from both lobes. The spacial separation of the cells on coverslips augments this apparent heterogeneity by preventing emission of light from adjacent cells to human tissue sections. In preliminary experiments, there appeared to be little, if any, heterogeneity of 125I-labeled mRNA content from cells in T lymphocytes, macrophages, and melanocyte heterogeneity appears to be due to large differences in POMC mRNA content. Further work is under way to determine the significance of this finding. This in situ hybridization method will complement tissue section methodologies, required for morphologic characterization, by providing better quantification of mRNA content of hormonally and developmentally regulated genes. This method should be equally useful for dissociated tissue and cultured cell systems.


The basis for selective vulnerability of motor neurons in amyotrophic lateral sclerosis is unknown. Investigation of motor neuron cell death has been hampered by poor yields and inhomogeneity of primary cell preparations. We have isolated 39 cloned cell lines from 6 series of neuroblastoma-spinal cord cell fusions in an effort to develop proliferating cell lines which express specific characteristics of a major population. NSC clones, but not N18TG2 cells, were investigated with current clamp microelectrode techniques. Resting potentials were observed at ~40mV. Depolarizing action potentials of ~35mV were elicited by anode breaks. NSC lines may provide reagents for dissection of motor neuron cell biology in a clonal system. (Supported by NS grants HD18438 and NS19397 (to RGA).)
420.9 IMMUNOHISTOCHEMICAL AND IMMUNOFLUORESCENCE LOCALIZATION OF ANGIOTENSIN GEN AND ANGIOTENSIN PEPTIDES IN PRIMARY FETAL RAT BRAIN CELL CULTURES. V. L. Leibel*, M. P. Prior, L. S. Calle**, Dept. of Pharmacology M-206, Univ. of Calif. San Diego, La Jolla, CA 92039.

While the existence of an endogenous brain angiotensin system (BAS) is no longer questioned, the specific cell type(s) which contain the angiotensin (ANG) peptides and/or angiotensinogen (ANGogen) the putative prohormone, are still controversial. To study the BAS at the cellular level we have established and maintained primary fetal rat brain cell cultures under serum-free conditions. The cell populations contain specific binding sites for the angiotensin peptides and secrete ANGogen into the medium. We have used cell specific markers to identify neurons and glia containing the angiotensin (ANG) peptides and/or ANGogen in co-localization studies. We used the avidin-biotin peroxidase (ABC-PO) method and the avidin-biotin alkaline phosphatase (ABC-AP) method as well as immunofluorescence on nonconfluent cultures. Cells were first stained for components of the angiotensin system and then with cell specific antisera. Using ABC-PO and ABC-AP immunohistochemistry we have found that ANGogen localizes primarily to neurons with however, some glia exhibiting specific immunoreactivity. Angiotensin peptides are found almost solely in neurons with few if any glial staining for the peptides. These results were confirmed in preliminary experiments using indirect immunofluorescence with either fluorescein-labelled or rhodamine-labelled secondary antibodies, while these serum-free cultures consist of a mixed population of neuronal cells, approximately 50-60% are neuronal. Of these, a surprising 20-30% appear to contain immunoreactive ANGogen while a smaller percent contain ANG peptides.

Conclusion: that in serum-free cultures of primary rat brain cells both the prohormone and peptide are localized largely in neuronal cells while the prohormone may also be contained in a subpopulation of glia. The latter may reflect uptake rather than de novo intracellular synthesis. This cellular system will be valuable for studies of regulation of the brain angiotensin system. (Supported by AHA California Affiliate #86-511 and HL 35018.)

420.11 STRUCTURAL AND FUNCTIONAL CONSEQUENCES OF PC12/ADRENAL MEDULLARY ENDOTHELIAL CELL INTERACTIONS THAT ARE MEDIATED BY SPECIFIC CELL SURFACE PROTEINS.


Adrenal chromaffin cells, a common model for studying neurosecretion, are in situ juxtaposed to capillary endothelial cells. Interactions between these two cell types might therefore be of importance for regulating the secretory functions. We have previously shown (J.Cell Biol. 103, 212a) that in vitro PC12 cells, a noradrenergic tumor cell line, specifically adhere to capillary endothelial cells, isolated from bovine adrenal medulla (BAME) cells. We have further characterized these interactions and report here on their structural and functional consequences. PC12/endothelial cell adhesion was insensitive to calcium levels above 1 mM in the growth medium and inhibited by serum proteins. Adherence of PC12 cells to endothelial cells monolayers (grown on 24 well plates) was independent of the cell density, provided more than 7x10^4 PC12 cells/ml were used.

Within one hour following plating onto BAME cells, PC12 cells were found to release augmented levels of [3H)-norepinephrine and vasopressin, stimulated with either acetylcholine, potassium or barium. PC12 and BAME cells in co-culture mutually inhibited their respective growth rates. PC12 cells, grown on endothelial cell monolayers, also lost their responsiveness to nerve growth factor. This is consistent with our previous suggestion, based on the elevation in the met-enkephalin contents in PC12 cells, that upon co-culture with the BAME cells, PC12 cells might reroute their differentiation towards a more chromaffin-like state.

PC12/BAME cell adhesion was selectively inhibited by trypan sensitive protein toxins isolated from proteoliposomes derived from cell surface extracts. These extracts, containing enriched amounts of a small fraction of plasma membrane proteins, were used to raise polyclonal antibodies. The purified IgG fraction specifically bound to membranes of PC12 cells and endothelial cells, respectively. Preliminary data also indicate that the anti-BAME-extract antibodies recognize one single ~62 kD protein. By contrast the antibodies raised against PC12 extracts reacted with several PC12 cell surface proteins. Currently we are characterizing those molecules that mediate specific interactions between PC12 and endothelial cells.

420.10 CALCIUM TRANSFER ACROSS THE ENDONEURIAL CAPILLARIES OF FROG SCIATIC NERVE. H. Levitan, K.C. Wadhwani and S.I. Rapoport. Lab. of Neurosciences, NATIONAL INSTITUTE ON AGING, NIH, Bethesda, MD 20892.

In view of the critical role that Ca plays in modulating many functions of vertebrate peripheral nerve, we studied properties of the Ca transfer across the endoneurial capillaries of female frogs, R. pipiens. An in situ perfusion technique and an in vivo i.v. bolus injection technique were used. For in situ perfusion, the vasculature of the hindquarters of an anesthetized frog was perfused with Ringers solution containing 45Ca, and 3H-inulin was used as a vascular marker. After perfusing for 5, 10 or 20 min, the sciatic nerve was exposed and desheathed. For the in vivo j.v. bolus injection technique, Ringers (0.2 ml) containing 45Ca and 3H-inulin was injected as a bolus into a left cutaneous vein. After 5 min, the hindquarters of the animal were severed from the body. In both techniques, the permeability-surface area product (PA) of 45Ca at endoneurial blood vessels was calculated from the amount of radioactivity in the desheathed segment, corrected for the contribution of radioactivity via the perineurial route. The PA of 45Ca, calculated from both techniques, equaled 4.4 x 10^-5 ml/sec.-1.g.-1 wt wt, and was independent of the [Ca^2+] in the perfusate between 0.18 and 18 mM. The half time (t1/2) for endoneurial Ca to equilibrate with plasma Ca was calculated to be about 75 min. The low, concentration-independent permeability of the endoneurial capillaries of frog sciatic nerve to Ca probably limits significant rapid changes in the Ca content of the nerve endoneurium during transient changes of plasma Ca, but does not alter steady-state responses. The results are consistent with the absence of homeostasis of whole nerve Ca during chronic hypo- and hypercalcemia (Wadhwani et al., Soc. Neurosc. Abstr. 12:1260, 1986).

Recent studies suggest that the axonal cytoskeleton has a more complex organization than previously recognized. The existence of a stationary non-uniform cytoskeleton as well as a moving component (Nixon and Logovinsky, 1986) implies that the interaction of cytoskeletal elements, its modification may be particularly relevant. Because of relatively unmodulated and exclusively in perikarya, a modified NFP-H is predominantly axonal. We report that at least four NFP-H variants are found within the axons of a single neuron type. These isoforms are generated by phosphorylation and appear to serve different functions.

Protein-labeled microtubules were isolated by subjecting mice intravitreally with radiolabeled microtubules and labeled NFP-H variants. The four variants exhibited nearly identical 2-0 coefficients and peptide maps. Phosphorylation is the likely basis of the two NFP-H variants predominating in the stationary cytoplasmic component. A monoclonal antibody recognizing a phosphate-dependent site was raised to this 160 Kd band. In vivo, labeled NFP-H variants were found in the axonal cytoskeleton and appeared to serve different functions.


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421.5 ACTIVATION OF RAT BLOOD PLATELETS PRODUCES A CALPAIN-MEDIATED DEGRADATION OF A BRAIN SPECTRIN-LIKE PROTEIN. M. Baudry, Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717.

When stimulated by a variety of agonists, blood platelets exhibit a variety of structural modifications affecting the cell cytoskeleton and the distribution of cell surface receptors. We previously proposed that a variant of this underlying mechanism operates in brain dendritic spines and is responsible for modification in synaptic structure and function elicited by brief periods of high frequency stimulation. We particularly suggested that activation of a calcium-dependent protease (calpain) and the resulting degradation of fodrin in brain spectrin was a general mechanism by which cells modify their structures and functions in response to external stimuli. In the present study we used a polyclonal antibody against brain spectrin to investigate the existence of the calpain/spectrin interaction in blood platelets.

Rat blood platelets exhibit a brain spectrin-like protein which migrates on SDS-polyamylamide gels between the actin-binding protein and spectrin and is responsible for modification in synaptic structure and function elicited by brief periods of high frequency stimulation. We particularly suggested that activation of a calcium-dependent protease (calpain) and the resulting degradation of fodrin in brain spectrin was a general mechanism by which cells modify their structures and functions in response to external stimuli. In the present study we used a polyclonal antibody against brain spectrin to investigate the existence of the calpain/spectrin interaction in blood platelets.

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Investigation of the physiological and chemosensitive properties of developing neurons in tissue culture is hampered by the heterogeneity of cell types. In the absence of selective antibodies or distinctive morphology, it is difficult to identify subpopulations of cell structure. (Supported by grant BNS 81-12162 to MB and AFSOR-86-0099 to GL."


The 55 kDa calmodulin protein is also present in skeletal muscle but not in non-excitable tissues. A distinct, immuno-crossreactive calmodulin binding protein was identified in the electric organ, heart, brain and spinal cord. Rat blood platelets exhibit a brain spectrin-like protein which migrates on SDS-polyamylamide gels between the actin-binding protein and spectrin and is responsible for modification in synaptic structure and function elicited by brief periods of high frequency stimulation. We particularly suggested that activation of a calcium-dependent protease (calpain) and the resulting degradation of fodrin in brain spectrin was a general mechanism by which cells modify their structures and functions in response to external stimuli. In the present study we used a polyclonal antibody against brain spectrin to investigate the existence of the calpain/spectrin interaction in blood platelets.

Calcium is an important regulator of neuromuscular function. Calcimedin is the major intracellular mediator of calcium action. Calcimedin binding proteins, for whom many functions are known, are the targets of active calmodulin. These proteins convert the calcium signal into coordinated metabolic and transport responses such as those that maintain metabolism and secretion. In order to identify calmodulin-dependent pathways involved in neurotransmission, those which have remained undetected by previous methodologies, we have utilized the E. electricus electric organ. Our approach has been to purify and characterize calmodulin associated proteins from this calmodulin and synapse rich tissue. Calmodulin is a major protein in the electric organ. Localization studies using the PAP technique demonstrates that calmodulin is present in the electrocyte and associated nervous tissue of the electric organ. We have employed calcium dependent hydrophobic chromatography to investigate the presence of other calcium mediating proteins including the calcemeds. Calcemedin 67 Kd is expressed in the electric organ.

In order to evaluate the discrimination of the calcium signal through calmodulin we have utilized calmodulin-Sepharose chromatography to identify calmodulin target proteins. There are approximately twenty polypeptides from the electric organ which bind calmodulin in a calcium dependent manner. Two protein species, 55 kDa and 47 kDa, are the most abundant and possess the highest affinities for calmodulin. Each protein was purified and antibodies produced. Immunoblot analysis of fresh tissue revealed only the 55 kDa species. The 47 kDa species was shown to be generated by endogenous proteolysis during purification. The 55 kDa calmodulin protein is also present in skeletal muscle but not in non-excitable tissues. A distinct, immuno-crossreactive calmodulin binding protein was identified in the electric organ, heart, brain and spinal cord. Rat blood platelets exhibit a brain spectrin-like protein which migrates on SDS-polyamylamide gels between the actin-binding protein and spectrin and is responsible for modification in synaptic structure and function elicited by brief periods of high frequency stimulation. We particularly suggested that activation of a calcium-dependent protease (calpain) and the resulting degradation of fodrin in brain spectrin was a general mechanism by which cells modify their structures and functions in response to external stimuli. In the present study we used a polyclonal antibody against brain spectrin to investigate the existence of the calpain/spectrin interaction in blood platelets.

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**421.0 MODULATION OF RENAL PORE ACTIVITY BY L10 AND THE LUQ CELLS OF APLYSIA**


Although the abdominal ganglion of *Aplysia* has long been popular for studying the neurobiology of behavior, the behavioral functions of most of the larger cells in the ganglion remain unknown. Here we show that some of the cells used most extensively for cellular studies are involved in the control of renal function.

Renal excretion in *Aplysia* begins with the formation of an ultrafiltrate of blood across the wall of the heart. This fluid is swept into the renal sac through capillaries and it is this fluid which is further elaborated into urine by excretion and absorption across the epithelial sheet of the renal sac. Urine leaves the renal sac via the muscular renal pore located near the posterior base of the gill (Eales, 1921).

Neurons L10 and the 5 left-upper quadrant (LUQ) cells, L2-L6, are located in the abdominal ganglion. L10 has been shown by others to be cholinergic. More recently, our lab has been attempting to characterize the LUQs as being both glutaminergic and containing neuropeptide myomodulin (Alvizo et al., this volume). The function and the transmitter type of the LUQ cells have not been described, although neuron L5 has been suggested to be cholinergic. The present study characterizes the characteristics of a neuropeptide (Shyama et al., et al., 1986). Both L10 and cells L2-L5, L6 have been shown to possess endogenous bursting properties and L10 is known to inhibit the LUQs.

These neurons seem to be likely candidates to modulate renal function because: (1) L10 had been shown to increase heart rate in excised heart cell RB-HF; so it may increase the rate of renal filtration; (2) We have found that L10 and a sub-set of the LUQs send axons to the renal pore.

Four types of evidence support the hypothesis that these cells control pore opening: (1) Hyperpolarizing L10 by current injection elicits spontaneous firing reduces the frequency of pore opening from 5/6 to 2/6 (N=6; reduced preparation); (2) Firing L10 by current injection initiates pore opening and firing some of the LUQ cells initiates pore closing; (3) L10 spikes elicit 4-10 UPS in the spawning circuitry that causes pore closure; (4) Touching the pore trigger pore closure and inhibits L10.

To explore further the role of L10 and the LUQs in controlling pore activity, we have begun an ultrastructural survey of synaptic contacts on striolar and circular muscle fibers. So far it appears that the cell bodies of most of these cells can be seen to be part of a peripheral neural plexus containing various synaptic contacts. This plexus may form an anatomical substrate for presynaptic modulation of transmitter release onto the pore musculature.

**421.11 BRAIN ORGANIZATION OF MANDUCA Sexta STUDIED WITH MONOCLONAL ANTIBODIES**

Akira Hishinuma and John G. Hildebrand

Nervous systems of insects have been an attractive subject for immunocytochemical research. As part of our continuing studies on the development and adult organization of the Manduca nervous system, we have recently isolated four MAbs that recognize neural antigens in Manduca brain.

The MAbs 28H2, 22E11, 44.1, and 8C3, identified antigens specifically associated with neural components (SCb, SCa) convey the cytoplasmic matrix and components (FC) which are found in the central nervous system of Manduca sexta have been isolated by screening hybridoma culture supernatants and subsequently rabbit antisera. These MAbs were isolated by screening 1,105 hybridoma cell lines prepared by using mice immunized with CNS tissue containing radioactive SCa proteins. None of the MAbs identified antigens containing radioactive SCb proteins. Another MAb (22C10) identified polypeptide bands from tissue subjected to immunoadsorption with each of the four MAbs. Visualization of radiolabeled antigen was by gel electrophoresis followed by fluorography.

All four MAbs identified antigens specifically associated with single axonal transport rate-components, and contralateral control nerves, were removed and subjected to immunoadsorption with each of the four MAbs. Visualization of radiolabeled antigen was by gel electrophoresis followed by fluorography.

Assignment of these antigens or antigen subunits to specific subcellular systems is possible. Certain monoclonal antibodies (MAbs) identify nervous system components (SCb, SCa) convey the cytoplasmic matrix and major axonal transport rate-components is thought to convey a unique transport rate.

The current work was supported by a grant from the National Institutes of Health (NS 22402 and MH 39145). Supported by NSF Grant BNS 86-4145.

**421.10 AXONAL TRANSPORT OF NEURAL ANTIGENS CHARACTERISTIC OF SUBPOPULATIONS OF CNS NEURONS**

Richard F. Atkinson and C.A. Miller*

Dissertation, Columbia Univ.). As part of our continuing studies on the development and adult organization of the Manduca nervous system, we have recently isolated four MAbs that recognize neural antigens in Manduca brain.

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To explore further the role of L10 and the LUQs in controlling pore activity, we have begun an ultrastructural survey of synaptic contacts on striolar and circular muscle fibers. So far it appears that the cell bodies of most of these cells can be seen to be part of a peripheral neural plexus containing various synaptic contacts. This plexus may form an anatomical substrate for presynaptic modulation of transmitter release onto the pore musculature.

Two young adult female baboons (papio ursinus) were fixed by transeptal perfusion in Nembutal anesthesia for a combined Golgi/electro生理ological (EM) study. Identification of cells in the hippocampus proper and fascia dentata. After Golgi impregnation, gold-toning, and embedding in paraffin, sections were stained with fast green and Nissl stain. The brains were then stained with Cresyl violet and the fascia dentata was resected. The hippocampus was then sectioned in the coronal plane, and the sections were studied with light microscopy. The results suggest that these interneurons do not share the physiological characteristics of interneurons in the rat. They are not true intracortical neurons, and they are not the same as the interneurons in the monkey.

422.2 VISUALIZATION OF HIPPOCAMPAL SYNAPSES IN BRAIN SLICES USING VIDEO MICROSCOPY. T. Brown and J. K. K. Kennedy. Div. of Neurosci., Bearon Rm. Institute, City of Hope, Duarte, CA 91010.

Recent advances in video microscopy (Inoue, Video Microscopy, 1986) have already had a major impact on cell biology. We have been able to explore the applicability of analog and digital video enhancement techniques to various types of living brain slices, including the lateral geniculate nucleus and visual cortex, the cerebellum, and the hippocampus. Using both fluorescent dyes and neurophysiological techniques, we are currently testing the most promising of these techniques to visualize cellular structure within the hippocampal slices.


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Preganglionic neurons (PGN) in the sacral spinal cord are important to excretory and sexual functions. Our previous studies have suggested that different neuronal subtypes may be important for different excretory and sexual functions. In the present study, we have used intracellular injection of horseradish peroxidase (HRP) to label individual neurons. The present study was supported by grants from the National Institute of Child Health and Human Development.
422.5 ESTIMATING THE ELECTROTONIC STRUCTURE OF NEURONS WHICH CANNOT BE APPROXIMATED AS EQUIVALENT CYLINDERS. W. K. Jacob. Department of Anatomy, Rush Medical College, Chicago, IL 60612.

Facial motor neurons exhibit a transient chromatolysis and no neuronal cell loss following axotomy resulting in a relatively normal morphology by 30 days postoperative (dpo). This study examined somato-synaptic somato-bouton contacts on facial motoneurons following severance of the facial nerve with the purpose of further detailing the events during the retrograde response to injury. Two groups of six adult hamsters underwent axotomy and removal of a distal segment of the right facial nerve. The left nerves remained intact to serve as a control. Brains of each group were perfusion-fixed at 5 or 30 dpo. The facial nuclei were dissected out and further processed for routine ultrastructural examination.

At 5 dpo the neurons showed a morphology consistent with the chromatolytic response. In addition, synaptic bouton contacts were sparse and many thin axon collaterals were observed within the neuronal perimeter. At 30 dpo normal morphological features of the neuron were observed as well as a large number of thin processes. Astrocytic processes were still seen intervening between synaptic boutons and the periphery of the soma. A quantitative study was undertaken to assay the percent coverage of somal profile by synaptic boutons in normal and 30 dpo neurons. Preliminary results show that a normal facial motoneuron has 30% of the perimeter covered by boutons while at 30 dpo only 8.5% of the somal membrane has bouton contacts.

This quantitative study has also shown that the total perimeter (somal membrane) of normal neurons and 30 dpo neurons is the same, confirming that the decrease in percent coverage by synaptic contacts seen at 30 dpo is caused by fewer synaptic boutons present after axotomy. Axotomized facial motoneurons survive the injury even though appropriate peripheral reconnections are not made and synaptic bouton contacts are below normal.


Laryngeal and abducens motoneurones intracellularly recorded and labelled with HRP were reconstructed in 3D from high resolution measurements using a computer-aided microscope. Each dendrite was isolated by computer dissection and described by the following parameters: spatial projection, length, diameters, tapering, branching pattern, daughter branch ratio and branching power. A principal component analysis applied to the geometrical and the directional parameters demonstrated that the individual dendrites of a single motoneuron occupied definite territories and differed from each other by their geometry. In order to retain this multifactor and since the criteria for reducing the arborization to equivalent cylinders models were infringed, cable properties were computed by partitioning the dendrites into a series of contiguous segments short enough to be considered an inequivalent cylinder. The steady-state properties of each segment were combined to obtain the following parameters at any site of the dendrite: electrotonic distance, from the soma, input resistance, somatotopical and somatofugal voltage attenuation and charge transfer effectiveness ratio. Individual dendrites showed significant differences in their electrical behaviour. The distal branches displayed low charge transfer efficiency which was not compensated by the soma and/or the density of synaptic boutons as revealed by a quantitative ultrastructural analysis at their level.

Low attenuation pathways were revealed between distal branches suggesting the possibility of branch to branch interactions and local computations. Ultrastructural observations of close appositions between labelled dendrites and unlabelled dendrites or soma together with dendro-dendritic synapses suggested that the distal dendrites may be involved in local cell to cell communication.

422.7 ESTIMATING THE ELECTROTonic STRUCTURE OF NEURONS WHICH CANNOT BE APPROXIMATED AS EQUIValent CYLINDERS. W. K. Holmes and W. Rail. Mathematical Research Branch, MIDR, NIH, Bethesda, MD 20892.

The electrotonic structure of a neuron determines how effective synaptic inputs may be. Formulae for computing the electrotonic length, L, of a neuron (Rall, 1969, 1977) have been widely applied to a number of neuron types. However, these formulae are strictly valid only for neurons which can be approximated as an equivalent infinite sum of exponentials \( V(t) = \sum C_i \exp(-t/T_i) \). If \( L \), \( t_0 \), \( t_1 \), \( C_0 \), and \( C_1 \) are known for a neuron satisfying equivalent cylinder constraints with uniform \( R_m \), \( L \) can be correctly estimated from any of three different experimental ratios: \( t_0/t_1 \), \( C_0/C_1 \), or \( C_0/V(0) \). However, misapplication of the three simple formulae to neurons that are not equivalent to cylinders with uniform \( R_m \) yields the wrong result. For example, if the formulae are not valid in such cases. Examples are given where \( t_0 \), \( t_1 \), \( C_0 \), and \( C_1 \), and three values of \( t_0 \) are computed for cells with different geometries and soma shunt. Two cells are best represented by cylinders of unequal length, \( L_0 \) computed using \( t_0/t_1 \) may be greater than the sum of the actual \( L \) values of the two cylinders (see also Segov and Rall, 1983). Taper tends to increase \( t_0/t_1 \) and \( C_0/C_1 \) giving an under-estimate of the tapering, \( \Delta L \). When a large shunt exists at the soma, \( C_0 \) is very small making \( L_0 \) computed using \( C_0/V(0) \) or \( C_0/C_1 \) very large (with little regard for cell geometry). In this case \( t_0/t_1 \) may be a very poor estimate of the taper constant of dendritic membrane.

Although one can derive formulae for \( L \) for structures other than cylinders, the computation limits their usefulness. Instead, we solve the constrained inverse problem: given experimental parameter estimates of \( t_0 \), \( t_1 \), \( C_0 \), \( C_1 \), input resistance, soma membrane area, and an approximate simplified geometry, find (using suitable constraints) values for the soma and dendritic time constants, the L of each segment, and the dendritic to soma membrane area ratio. This has been done for cells represented as cylinders with a soma shunt using Newton's method. Resolution of model parameter values depends on the constraints chosen and on the accuracy of experimental parameter estimates.

(This work was supported in part by NS07845).

We are attempting to develop autoradiographic techniques to identify neurons capable of binding radiolabeled ecdysteroids in the nervous system of the moth Nanduca sexta. Individual abdominal ganglia are dissected and incubated in 3H-ecdysterone, a biologically active phytoecdysterone previously used to characterize ecdysteroid receptors in other tissues. Individual neurons are then identified by their specific response to several ecdysteroids. These experiments are designed to determine when neural estrogen binding sites appear during the development of the moth nervous system, including neuronal morphology, responses to peptide hormones, differentiation of post-embryonically-generated neurons, and cell death. Mapping ecdysteroid target cells in the developing nervous system of Nanduca sexta will guide future studies of the mechanism underly­ing the actions of these steroid hormones on specific, identified neurons.

Research supported by NIH grants NS-07597 (SEF) and NS-13079 (JWT).


We observed transitory elevations of CAR in cortical tissue in early gestation. The results indicate that estrogen binding sites appear in the central nervous system during early gestation in both early and late gestation. The development of estrogen binding sites in the central nervous system is similar to the development of estrogen binding sites in the peripheral tissues.

These data provide new information regarding the ontogeny of CAR in brain tissue of fetal rhesus monkeys. We observed transitory elevations of CAR in cortical tissue in early gestation which was controlled by T. This may be an important observation for understanding the cellular components of sexual differentiation of the primate brain.

[Supported by NIH 16022]

423.4 SELECTIVE ANDROGEN EFFECTS ON RAT SPINAL MOTORNEURON MORPHOLOGY AND CONNECTIVITY.  C.M. Kurz, C.J. Bowers, and D.L. Segalow, Dept. of Psychology, Indiana University, Bloomington, IN 47405.

In male rats, the spinal nucleus of the biceps femoris (SNB) projects to the perineal muscles bilobocervical (IC) and levator (LH), and the dorsolateral nucleus (DLM) projects to the inosculating (IC) muscles. Adult females have no perineal muscles; however, females treated prenatally with the androgen dihydrotestosterone (DHT) or testosterone (T) have shown development of the perineal muscles, which are now anomalously innervated by motorneurons in the DLM (Breedlove '86). In normal males the morphology of DLM and SNB motorneurons is significantly different from that of normal females (Arnold, '86). To investigate the factors which determine neuronal morphology and connectivity, we compared SNB and DLM motorneurons having either normal or hormonally altered specificity.

Timed pregnant rats (Sprague-Dawley) received DHT (2 mg/day) or oil, SC, from embryonic (E) days 17 to 22; at birth (E25) pups were cross-fostered to other lactating females. At 60 days of age DHT-females were given testosterone implants to increase muscle mass for reliable identification. Motorneurons were visualized by injecting 0.5 μl of cholera toxin (CIT) unilaterally into either the IC, LCA, or IC nucleus of DLM-females or normal males at E5-6 days of age (E6-8). After 48 hours cords were processed with TRP and the number, location, soma size, number and orientation of primary processes, and distribution and amount of dendritic arbor of L-FP-labeled cells were recorded.

In both groups virtually all labeled motorneurons were located in the DLM after IC injection, or the SNB after LA injection. After IC injection, 97% of labeled cells were in the SNB of males. In DHT only 97% were localized in the SNB whereas 90% were located in the DLM. In DHT-females these cells were localized in the DLM. In DHT-females the morphology of SNB motorneurons projecting to the LA (SNB-LA) was identical (76% of motorneurons in DHT-females showed similar process orientation, 2 per cell in 830 μm). DHT-IC motorneurons were larger in males, but SNB-LC motorneurons in DHT-females had smaller somas (890 μm²) and less per cell (1670 μm²) than SNB-LC motorneurons in DHT-females. In DHT-females were smaller than those of normal males, but SNB-LC motorneurons were larger in males, but SNB-LA motorneurons in DHT-females were smaller than those of normal females (890 μm²), number of processes (5.4 vs. 4.9), and arbor per cell (2700 vs. 1700μm²). DHT-IC and DHT-LC motorneurons in DHT-females were compared to SNB-LC motorneurons in the DLM-IC motorneurons had smaller somas (625 μm²) and fewer processes (4.6), oriented differently. Motorneurons more powerful regulators of movement by perirhinal targets and only partially affected by the local microenvironment of the spinal cord. Moreover, prenatal DHT treatment in females selectively influences motorneuron morphology and alters the connectivity of motorneurons projecting to only one of the SNB's normal targets. (Supported by NIH grant NS-25877)

A sexually dimorphic nucleus has recently been identified at the dorsal border of the preoptic and anterior hypothalamic areas (POA/AM) in the ferret (Tobet et al. Proc. Natl. Acad. Sci. 83: 1439, 1986). Some areas of neurons in the male nucleus (POA/AM) are larger than areas of cells in a corresponding region of the female although the pattern of sex differences in somal size and grouping extends to the dendritic structure of individual neurons, POA/AM tissue from adult male (n=5) and female (n=6) ferrets was processed using the Golgi-Cox procedure, sectioned at 120 μm, and counterstained with thionin. The animals were not castrated and had received no steroids for 23 (5 males), 3 females) or 5 days (2 females) prior to sacrifice. Multinuclear neurons with cell bodies in the VM-POA/AM or in the corresponding region of females were studied. A semi-automated microscope and computer system was used to trace dendritic branching of 19 male and 17 female neurons in three dimensions, and somal areas of these neurons were estimated from camera lucida tracings.

Dendritic density (determined by the number of intersections of dendrites with concentric spheres drawn around the cell body) and total dendritic length were both significantly greater for male neurons (mean ± SE; density: 93 ± 7 vs 65 ± 6 intersections, t(34)=2.85, p<0.01; length: 721 ± 46 vs 576 ± 57 μm, t(34)=1.98, p<0.05). In contrast, two measures of dispersion, mean distance from the cell body to dendrite tips (males: 101 ± 4, females: 98 ± 7 μm) and median radial distance (the distance from the cell body at which there are equal numbers of intersections proximally and distally: males: 65 ± 3, females: 60 ± 4 μm), were equivalent in males and females. Also, somal areas of the selected multipolar neurons were similar in males (143 ± 7 μm²) and females (145 ± 9 μm²).

Thus the extension of dendrites from the cell body in the dorsal POA/AM is similar in males and females, whereas dendritic length and density are greater in male neurons. These results suggest that the potential for synaptic contacts within a given radius from the cell body is greater in the POA/AM of males. It is notable that sex differences in dendritic morphology were found in neurons with equivalent somal dimensions. Future research will reveal whether steroid-environmental influences sexually dimorphic features of dendritic structure in either multipolar or other types of POA/AM neurons.

Supported by: HD-21094, HD-21033, and HD04941-01


Both estrogens and androgens have been shown to exert masculinizing influences upon CNS morphology and behavior in vertebrates. The motoneurons of the spinal nucleus of the bulbocavernousus (SNB) innervate the bulbocavernousus (BC) muscles in the perineum. Both the BC and its SNB cells are present in newborn female rats but die shortly thereafter. The involution of the SNB system can be prevented with neonatal testosterone or dihydrotestosterone propionate (DHTP) treatment, but not by estradiol benzoate (EB) alone. Because it had not yet been established whether EB, in the presence of androgen could masculinize the SNB system, we treated newborn female rats with EB which did not spare SNB motoneurons from death (Juraska et al. Brain Res. 295:27, 1984) and likely to be independent of the effects of EB. Neonatal EB did significantly increase the adult size of SNB somas and nuclei (p<0.01). There was no significant interaction of EB and DHTP for any of the measures, indicating that the adult SNB system is controlled by factors unresponsive to neonatal sex hormones. Neonatal EB and DHTP permanently masculinized both the number and the size of SNB motoneurons (p<0.01). EB had no effect on the number of SNB cells other than the SNB, suggesting differential influence on SNB size independently. Since in adulthood SNB motoneurons accumulate androgens but not estrogens, and because EB is known to masculinize the developing brain sites, these two metabolites of testosterone may normally act in concert via different mechanisms (perhaps at different sites) to increase SNB size. Therefore, we attempted to test this hypothesis by providing an excess of testosterone to male rats during their early postnatal development and examining the visual cortex, an area that we have demonstrated to be sexually dimorphic (Juraska, Brain Res. 295:27, 1984) and likely to be influenced by neonatal testosterone.

Male rats were injected with 1000 pg of testosterone propionate in oil on days 3, 5 and 7. Control littermates of both sexes were injected with oil alone on the same days. At 23 days of age, all rats were perfused with paraformaldehyde, their brains were frozen sectioned at 40 microns and stained with cresyl violet. The visual cortex (areas 17 and 18) was examined in rats from four litters (26 animals) at one anterior and one posterior level. Four measurements of cortical depth and individual layer depth were taken at intervals scaled to the size of each brain in both hemispheres. There were no statistically significant effects of excess testosterone in the cortical depth measures, nor were there behavioral differences between male and female rats. This data do not support the Geschwind and Behan hypothesis. We are currently examining cell density in the visual cortex and extending our studies to other cortical areas.

Supported by: NIMH


We have previously demonstrated that female exhibit dendritic plasticity in hippocampal dentate granule neurons in response to differential environments, while males do not (Juraska et al. Proc. Natl. Acad. Sci. 83:7317, 1985). Sex differences also were found within each environment such that males had more dendritic material than females following rearing in a complex environment such that males had more dendritic material than females when raised in an isolated environment, while females had more dendritic material than males following rearing in a complex environment. In the present experiment we investigated whether testosterone (developmental and post-puberal) was responsible for the male pattern of results.

Male rat pups were castrated or sham operated within 6 hours of birth and returned to their mothers. At weaning, littermate sets of these rats were assigned to either an isolated (no enriched environment) or a relatively complex environment in which rats of each hormonal condition were separately group housed with objects that were changed daily. After a month of differential rearing, the rats were sacrificed; the brains were Golgi-Cox stained and nuclei were drawn with a camera lucida, their cross-sectional area (p<.01). There was no significant interaction of EB and DHTP for any of the measures, suggesting that the adult SNB system is controlled by factors unresponsive to neonatal sex hormones. Neonatal EB and DHTP permanently masculinized both the number and the size of SNB motoneurons (p<0.01). EB had no effect on the number of SNB cells other than the SNB, suggesting differential influence on SNB size independently. Since in adulthood SNB motoneurons accumulate androgens but not estrogens, and because EB is known to masculinize the developing brain sites, these two metabolites of testosterone may normally act in concert via different mechanisms (perhaps at different sites) to increase SNB size. Therefore, we attempted to test this hypothesis by providing an excess of testosterone to male rats during their early postnatal development and examining the visual cortex, an area that we have demonstrated to be sexually dimorphic (Juraska, Brain Res. 295:27, 1984) and likely to be influenced by neonatal testosterone.

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Supported by: NIMH
423.9 POSTNATAL ONTOGENY OF SEROTONIN AND SUBSTANCE P IN THE SEXUALLY DIMORPHIC CERAMATOSTIC NUCLEUS, M.A. Konagaya, B.M. Newton & R.M. Small. Departments of Neurology and Medicine, University of Rochester/Monroe Community Hospital, Rochester, NY 14603.

The rat ceramatosetric nucleus lies in the LI-2 ventral spinal gray. In males this nucleus innervates the cremaster muscle; in females the peripheral target is unknown. Previous serotonin (5HT) and substance P (SP) immunohistochemical studies revealed substantial sexual dimorphism, with males containing greater or almost exclusive amounts of immunohistochemically dense 5HT and SP in cremaoteric, bulbocavernosus and corporal system structures during development.

Five-HT and SP fibers are barely discernable in the cremastatic nucleus. At P10 a dramatic increase in 5HT fibers is observed with the fibers approaching adult numbers. At P20-40 the density and distribution of 5HT fibers in the ceramatosetric nucleus resembles adult densities.

These data indicate that SP and 5HT fibers develop independently of each other in the ceramatosetric nucleus. However, the appearance of each transmitter may be related to postnatal testes descent and the regulation of testosterone temperature in the scrotal region. This research was supported by NIH grant NS12247 and A.M.


The rat ceramatosetric nucleus lies in the LI-2 ventral spinal gray. In males this nucleus innervates the cremaster muscle, and may play a role in testes temperature regulation; in females the peripheral target is unknown. Immunohistochemical and morphological studies reveal sexual dimorphism of this nucleus with males containing greater amounts of HIC demonstrable SHT and SP in the ceramatosetric nucleus and 57% more ceramatosetric neurons (Anat. Embryol. 170:171,8; Brain Res. 301:24, ’84). Brain Res. Bull. 156/6/85, Neurosci. Lett. 64:175, ’86). The present studies were performed to determine: 1) if the amount of SHT, and a metabolic product 5-hydroxyindoleacetic acid (5-HIAA) in the cremasteric nucleus is sexually dimorphic as compared to IHC evidence; 2) if ceramatosetric SHT and 5HT levels are dependent upon reproductively unprimed males; and 3) if sexually dimorphic differences exist in SHT and 5HT levels in cold stressed rats. Male and female rats (n=7; P90) were killed with a 14 day head低温 and the 11th day levels rapidly removed and frozen on dry ice. The ventral gray pool was micro-punched, placed in 5% perchloric, postcomitally disrupted, and the serotonin and 5HT fractionated by HPLC with coulometric detection. In addition, littersmates (n=7) were used, as controls, and no significant differences were observed. Male and female rats were expressed three ways. Results expressed per gender indicates the male ceramatosetric nucleus contains more SHT and 5HT (5320pg/mm2 x 3.5±S.E.M.) than females (4240±283 and 1570±145pg); when expressed as pg/mm2 protein the male cerebrocystic nucleus contains more SHT and 5HT (111±9 and 36.9±5.9pg) than males (71±7 and 23±2.0pg). However, if the difference in neuron number, which may reflect protein content of the nucleus, is removed the male values for SHT and 5HT are increased by 57% then female rats and males contain 111±9 and 110±11pg (corrected value) of SHT and 5HT. A number of SHT and 5HT fibers are present in the cremastatic muscle of the male vs the rat muscle. This research was supported by NIH grants NS19790 and the Sloan Foundation.

The spinal nucleus of the bulbulo-sacrococcygeus (SNB) is a sexually dimorphic group of motoneurons in rat spinal cord that innervates perineal muscles. Male SNB motoneurons are more numerous than female neurons. These cellular attributes are regulated developmentally by androgen. Androgen treatment of neonatal females permanently masculinizes (increases) the size and number of SNB neurons.

The purpose of this study was to determine the end of the critical period for this androgenic regulation of SNB soma size in females.

Female Sprague-Dawley rats each received three injections of testosterone propionate (TP, 1 mg/day) or saline from postnatal days 1-5 or 7-11 (p<.001). Animals were sacrificed on postnatal day 21, and their spinal cords were sectioned at 50 um and stained with thionin. The cross-sectional areas of 25-32 SNB somas per animal were measured using a computerized digitizing tablet interfaced with the microscope. All groups were compared with normal males and females that received no neonatal treatment but the same adult treatment as the other groups.

As reported previously (Breedlove and Arnold, 1983), neonatal TP injections masculinize the soma size of the male SNB motoneurons if given at postnatal days 1-5 or 7-11 (p<.001). Differences found after this period do not have a significant effect (p>.05).

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This data indicate that the critical postnatal period for permanently increasing female soma size ends around the second week of postnatal life. This information may help delineate which cellular events underlie the critical period for postnatal androgenic regulation of cell size. Supported by NIH grant HD-05021.


The sexually dimorphic nucleus of the preoptic area (SDN-POA) of the rat is larger in volume in males than in females, is hormone sensitive and in the male is stimulated by exogenous androgen. The central part of the SDN-POA (SDN-POA) is more responsive to androgen than the posterior SDN-POA.

These data imply that the critical postnatal period for permanently increasing female soma size ends around the second week of postnatal life. This information may help delineate which cellular events underlie the critical period for postnatal androgenic regulation of cell size. Supported by NIH grant HD-01182.

423.15 DURATION OF THE ANDROGEN SENSITIVE POSTNATAL PERIOD FOR DIFFERENTIATION OF THE SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA IN MALE AND FEMALE RATS, R. W. Sheets*, J. E. Shryne* and R. A. Gorski. (SPON: D. E. Fleming), Department of Anatomy and Laboratory of Neuroendocrinology, UCLA School of Medicine, Los Angeles, CA 90024.

This laboratory has demonstrated the existence of a marked sex difference in the volume of an intensely staining component of the preoptic area of the rat brain. This volume of the preoptic area, which is called the sexually dimorphic nucleus of the preoptic area (SDN-POA), is several fold larger in adult males than in adult females. The volume of the SDN-POA can be influenced significantly by the hormone milieu during early postnatal life. We used a thymidine incorporation technique to identify a critical period for the effects of either of these two molecules on muscle maturation or later sexual performance.

Selective prenatal treatment of pups with nicotine or ACTH at 0.25 mg/kg/2x day, i.p.---or nicotine---0.25 mg/kg/2x day, i.p.---nicotine during either of three gestational periods---G3-8; G9-13; G16-20---selectively manipulates the prenatal environment, profoundly affecting both early muscle maturation and later sexual performance.

Our previous studies have shown that the administration of either ACTH or nicotine alters both the maturation of the extensor digitorum longus (EDL) muscle-penile nerve complex and the development of the sexually dimorphic nucleus of the preoptic area. These studies demonstrate that androgen and estrogen play a critical role in the differentiation of the SDN-POA. These data suggest that the critical period for the effects of either of these molecules on muscle maturation or later sexual performance is sensitive to androgen action up through postnatal day 5 and to estrogen action up through postnatal day 7.

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Supported by the Council for Tobacco Research, and NIH grant 5T32HD-07282.


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Supported by HD-01182 & HD-07228.
424.1 MOLECULAR CHARACTERISTICS OF SOMATOSTATIN RECEPTORS IN PITUITARY CELLS. Kyriakos Thermis and Terry Reisine, Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA.

Somatostatin (SRIF) is a hypothalamic peptide which inhibits the release of a variety of hormones from the anterior pituitary. To study the molecular mechanism by which SRIF regulates pituitary activity, we have used a pituitary adenocarcinoma, GH3, which secretes ACTH, and GH, which produces growth hormone and prolactin have been highly useful. Functional studies (inhibition of hormone release, CAMP formation, and calcium influx) show that the pituitary cell lines are different. Such functional differences may indicate that the SRIF receptors in the two cell lines are subtypes. SRIF receptor subtypes have been proposed to exist in brain, pituitary, pancreas as well as other tissues. To ascertain whether these functional differences are the result of structural variations in the SRIF receptor we have determined the physical properties of the SRIF receptor in GH3 and AtT-20 cells. SRIF receptors were photolabeled with [125I]SRIF-2B, [125I]TrpB-SRIF and [125I]I251CGP-29392 and the labeled membranes were analyzed by PAGE. Analysis of the [125I]CGP-29392 labeled proteins by two-dimensional PAGE also revealed a single receptor in both cell lines with an apparent molecular mass of 55±5 kD. SRIF, SRIF-2B, TrpB-SRIF and I251CGP-29392 all bind to the same receptor. This result is further supported by the ability of forskolin stimulated adenyl cyclase activity in AtT-20 cell membranes to be reduced following TrpB-SRIF treatment. SRIF and GTP inhibition of forskolin stimulated adenyl cyclase activity takes longer (1/2 30 min) to occur but is more rapidly reversible (1/2 30 min).

424.2 MOLECULAR MECHANISMS OF SOMATOSTATIN INHIBITION OF cAMP-DEPENDENT AND cAMP-INDEPENDENT CALCIUM INFLUX IN THE CLONAL PITUITARY TUMOR CELL LINES, AT-T-20 AND GH3. Hunq-Li Wang* and Terry Reisine, Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia PA.

Somatostatin (SRIF) is a potent and effective inhibitor of hormone release from the anterior pituitary. One mechanism through which SRIF blocks hormone release is by inhibiting cAMP-dependent adenyl cyclase activity. Another mechanism is the increase voltage-activated calcium influx. Two of these responses to SRIF are independently controlled. These functional responses in the pituitary cell lines are distinct. In GH3 cells the population of SRIF receptors are coupled to adenylate cyclase and calcium channels. To investigate this question the characteristics of SRIF inhibition of calcium influx stimulated by corticotropin releasing factor (CRF), an activator of adenylate cyclase and K+ a membrane depolarizing agent, in AtT-20 cells, a clonal cell line of the pituitary, were assessed. Changes in cytosolic calcium levels were measured using the fluorescent probe Quin 2. Both CRF and K+-induced cytosolic calcium levels were blocked by SRIF. These results suggest that the stimulatory GTP activators of adenylate cyclase, such as forskolin, to increase cytosolic calcium levels in these cells is also blocked by SRIF. The ability of somatostatin analogues to inhibit the forskolin stimulated calcium influx is different in GH3 cells than their inhibition of SRIF stimulated calcium influx in At-T-20 cells. SRIF inhibition of forskolin stimulated calcium influx is not transitory as in the At-T-20 cells. These results are consistent with the hypothesis that the SRIF receptors on somatostatin and corticotropin (At-T-20) may be functionally distinct subtypes. Supported by NIH grants DK34782 and DK34782 and a Grant-in-Aid from the AMH.

424.3 MOLECULAR MECHANISMS OF SOMATOSTATIN DESSENSITIZATION. Marilyn Noell* and Terry Reisine, Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia PA, 19104.

Somatostatin (SRIF) inhibits the release of adrenocorticotropin from the anterior pituitary cell line At-T-20. This inhibition is transitory due to desensitization of the SRIF receptors. SRIF desensitization appears to result from an inability of the SRIF receptor to couple to the catalytic subunit of adenylate cyclase. One mechanism through which SRIF inhibits adenylate cyclase activity is by desensitization. Two types of somatostatin receptors exist. One is of high affinity and the other is of low affinity. SRIF receptors in GH3 and At-T-20 cells and is functionally active but appear to have the same primary structure. Variations in posttranslational processing may therefore account for the differences which can be confirmed in the functional differences. Such processing may be important for creating SRIF receptor subtypes in normal tissue, since preincubation of the membranes suggests that the catalytic subunit of adenylate cyclase is different in brain, pituitary and pancreas. Supported by NIH grants DK34782 and DK34782 and a Grant-in-Aid from the AMH.

424.4 EFFECTS OF SOMATOSTATIN ANALOGUES ON DEPHOSPHORYLATION OF THE PRESYNAPTIC PROTEIN, B-50. M.R. Pilsang*, D.H. Coyle* and L.A. Dokas, Departments of Biochemistry and Neurology, Medical College of Ohio, Toledo, Ohio 43699 and Department of Medicine, Tulane University, New Orleans, LA 70112.

Two types of somatostatin receptors exist. One is of high affinity and the other is of low affinity. SRIF receptors in GH3 and At-T-20 cells and is functionally active but appear to have the same primary structure. Variations in posttranslational processing may therefore account for the differences which can be confirmed in the functional differences. Such processing may be important for creating SRIF receptor subtypes in normal tissue, since preincubation of the membranes suggests that the catalytic subunit of adenylate cyclase is different in brain, pituitary and pancreas. Supported by NIH grants DK34782 and DK34782 and a Grant-in-Aid from the AMH.

Peptide III and [Nal]-somatostatin, which SRIF blocks hormone release is by inhibiting adenyl cyclase activity. Another mechanism is the increase voltage-activated calcium influx. Two of these responses to SRIF are independently controlled. These functional responses in the pituitary cell lines are distinct. In GH3 cells the population of SRIF receptors are coupled to adenylate cyclase and calcium channels. To investigate this question the characteristics of SRIF inhibition of calcium influx stimulated by corticotropin releasing factor (CRF), an activator of adenylate cyclase and K+, a membrane depolarizing agent, in AtT-20 cells, a clonal cell line of the pituitary, were assessed. Changes in cytosolic calcium levels were measured using the fluorescent probe Quin 2. Both CRF and K+-induced cytosolic calcium levels were blocked by SRIF. These results suggest that the stimulatory GTP activators of adenylate cyclase, such as forskolin, to increase cytosolic calcium levels in these cells is also blocked by SRIF. The ability of somatostatin analogues to inhibit the forskolin stimulated calcium influx is different in GH3 cells than their inhibition of SRIF stimulated calcium influx in At-T-20 cells. SRIF inhibition of forskolin stimulated calcium influx is not transitory as in the At-T-20 cells. These results are consistent with the hypothesis that the SRIF receptors on somatostatin and corticotropin (At-T-20) may be functionally distinct subtypes. Supported by NIH grants DK34782 and DK34782 and a Grant-in-Aid from the AMH.

Ala-Gly-Cys-Lys-Asn-Asp-Phc-[D-Trp]-Lys-Thr-Phc-Thr-Ser-Cys

(D-Trp)3-somatostatin

(D)-Phe-Cys-Phe-[D-Trp]-Lys-Thr-Cys-Thr-O

SMS 201-995

(S)-Phe-Cys-Thr-(D)-Trp-Lys-Val-Cys-Thr-amide

[Peptide III]

[Nal]-Cys-Tyr-(D)-Phe-Cys-Thr-O

(nal)-somatostatin

[Ur]-somatostatin


Radioligand binding and functional assays were employed to characterize a somatostatin (SS) receptor in the murine neuroblastoma clone NIE-115. [125I]-Tyr3-Somatostatin-14 bound specifically to a single class of sites in membranes prepared from NIE-115 cells (KD=0.03 ± 0.08 M; Bmax=1.0 ± 0.05 pmol receptor/cell). Competitive binding studies with cyclon-N-Me-ALA-Tyr-D-Trp-Lys-Thr-Phe (L63,656) did not indicate the presence of putative SS receptors subtypes found in rat brain membranes. In intact cells, SS-14 and its analogs decreased by 30-50% the levels of cyclic AMP induced by forskolin, prostaglandin E1 (PGE1) or vasodepressor intestinal polypeptide. The BCS values for SS-14, 0.01 M, and the IC50 for the loss of response where comparable to their respective binding constant obtained by competitive binding studies. The effect of SS-14 on cyclic AMP levels was not affected by muncastatin, histamine H1 or opioid receptor blockade. SS-14 did not inhibit inositol phospholipid hydrolysis, inositol phosphates. Binding of [125I]-Tyr3-Somatostatin-14 to cell membranes was reduced by guanine nucleotides. Pertussis toxin treatment of cells substantially reduced [125I]-Tyr3-Somatostatin-14 binding and the ability of SS-14 to activate phospholipase C. The SS receptor system in NIE-115 cells may serve as a model for one of the putative subtypes of SS receptors found in brain.


Decreased regional brain concentrations of somatostatin (SRIF), a tetracacid peptide, have been demonstrated in Alzheimer's disease by several research groups including our own (Soc. Neurosci. Abstr. 9:1052, 1983). Pretreatment with other neuropeptide systems, e.g. corticotropin-releasing factor (CRF), SRIF receptor antagonists, however, did not affect the number in the CNS of patients with Alzheimer's disease, in spite of the decrease in SRIF concentration itself (Neal et al., Science 229: 289-291, 1985). We have now scrutinized the SRIF binding assays to determine whether a decrease in SRIF availability results in the expected downregulation of the number and/or function of SRIF receptors, or whether the presently reported results are artefactual. We initiated these studies with a systematic evaluation of the variables of the receptor binding assay: method of membrane preparation, addition of protease inhibitors and other components to the incubation medium, centrifugation technique for separating free versus membrane bound radioactivity, and the use of non-frozen versus frozen tissue preps.

In determining the effects of freeze-thaw cycles on the SRIF receptor we used frontal cerebral cortex of rats as a model. Since one half was frozen and the other half was homogenized and used in a binding site saturation analysis using [125I]-Tyr3-SRIF to determine binding site kinetics. We further prescreened a panel of SRIF antagonists to determine homogeneity and froze it at -70°C for later analysis. The frozen portion of the rat cortex and the frozen homogenate were then examined after 24 to 48 hours of storage at -70°C. We observed a highly significant 40% reduction in the number of binding sites (Bmax) in the frozen tissue in contrast to 6% reduction in the Bmax of the frozen homogenate. The apparent affinity constant (Kd) was not affected by freezing and thawing. Thus present results obtained in frozen brain tissue must now be regarded as problematic.

Human cortical tissue obtained by rapid autopsy (within 30 to 60 minutes after death) is now being used to examine whether compensatory increases in SRIF receptor number occur in Alzheimer's disease. (Supported by MH-40524, MH-39415, and NIA AG-05128).


Murine neuroblastoma clone NIE-115 possesses receptors which specifically bind the tridecapeptide neurotensin (NT) and mediate the formation of intracellular cyclic GMP and the stimulation of inositol phospholipid hydrolysis. These cells also rapidly degrade NT in a sequential fashion (Soc. Neurosci. Abstr. 12:762, 1986). To study the effect of prolonged exposure of cells to NT on subsequent neurotensin receptor-mediated intracellular cyclic GMP formation, conditions were employed in which NT degradation was prevented: the incubation of 10-5 M NT with 0.1 x 106 intact cells in a phosphate-buffered saline solution at 37° with incubation times to the right of the desensitization of the treated cells to respond to NT stimulation was then measured following exposure of intact cells to neurotensin. The time course of this desensitization following exposure of intact cells to NT for only 5 min. This desensitization was rapid and NT demonstrated no decrease in their cyclic GMP production upon stimulation of intact NIE-115 cells for increasing lengths of time caused time-dependent shifts in the IC50 of the response. Together these results indicate that the NT binding activity of DYN and L-156,903 competition were similar (IC50 ~ 25 nM) whereas the low affinity components differed (IC50 ~ 5 nM). Competitive studies with L-156,903 reduced binding potency. [3H]Neurotensin (NT) binds to rat brain and uterus in vitro in a complex manner, exhibiting similar 'high' (Kd ~ 3 nM) and 'low' (Kd ~ 5 nM) affinity binding components. Competitive studies with NT fragments suggest a fundamental similarity in the recognition pattern of the NT binding sites. (Endo. Soc. Abstr. 73:1), 1987). In the present studies, however, using dynorphin A-1-13 (DYN), L-156,903 (N-[oxy-3-(1'-[3H]phenylalanine-10-yl) propyl]-arginyl-l-prolyl-tyrosine]), and L-156,903, clear distinctions in the NT binding sites of brain and uterus emerged.

3. Brain. Both DYN and L-156,903 inhibited nearly equally [3H]NT (1 nM) binding to rat brain membranes producing biphasic, shallow (mM, 0.5-0.6) competition curves indicating widely divergent affinities for the two [3H]NT binding sites. The high affinity components of DYN and L-156,903 competition were similar (IC50 ~ 25 nM) whereas the low affinity components differed (IC50 ~ 25 nM). Structure activity studies with dynorphins and L-156,903 indicated that the NT binding activity of DYN was due to the non-opioid C-terminus. Substitution of His for Arg or Tyr for Phe for L-156,903 reduced binding potency.

Inclusion of L-156,903 or DYN in [3H]NT saturation binding assays at concentrations near the IC50 values at points of their competition curves, reduced dramatically [3H]NT binding to the 'low' affinity (50 nM) NT site with comparatively little effect on the 'high' affinity (0.3 nM) site. Similar results were found with levcabastine (5 \mu M), a compound which selectively blocks the 'low' affinity binding site for NT (Schleedeberg's Arch Pharm 333: 400, 1986). Levcabastine (5 \mu M) completely eliminated the high affinity binding component of DYN and L-156,903, leaving only the low affinity component (IC50 ~ 20.3 \mu M). This compound had no effect on the 'low' affinity NT site.

4. Uterus. DYN and L-156,903 were considerably weaker (IC50 ~ 100 \mu M) in competing with [3H]NT (5 nM) binding to rat uterine membranes. Similarities were noted in that concentrations up to 100 \mu M were completely ineffective for the 'low' affinity NT site.

Thus these results demonstrate significant differences in the binding profile of the 'low' affinity NT site between rat brain and uterus.
424.9 MODULATION OF HYPOPHYSAL CHOLECYSTMOKIN RECEPTORS WITH CHAMPS IN MONOCULAR ACTIVITY. R.C. Gober, R.R. Hill and J.H. Hughes. University of Washington, School of Medicine, Seattle, WA.

Cholecystokinin 28-33 (CCK) is found in high concentrations in the hypothalamus, where it is distributed amongst a number of discrete nuclei. CCK receptor distribution in the hypothalamus correlates well with peptide distribution, with high densities observed in the ventromedial, dorsomedial, paraventricular (PVN) and suprachiasmatic nuclei (SCN). Clark et al., 1987. The presence of CCK receptors in the PVN and SCN suggests a role for CCK in neurosecretory processes and has prompted the present investigation into the function of these receptors. Hence, PVN and SCN CCK receptor distribution and density were determined by autoradiographical techniques in saltloaded and homozygous Brattleboro rats. Sections were subsequently washed, dried and apposed to Ultrofilm for 7-14 days, followed by development of the film in Kodak B. The resulting autoradiograms were used to produce black and white images of receptor distribution, and analyzed by computer-assisted image analysis to quantify receptor density.

11C-CCK receptor binding was greatly elevated in the PVN and SCN of the saltloaded SD rat compared to control animals. Similarly, the hypothalamic Brattleboro rat had significantly greater levels of PVN and SCN 11C-CCK receptor binding compared to the heterozygous Brattleboro rat and LE controls. Detailed distribution studies revealed that this increase in 11C-CCK receptor binding was localized only to the magnocellular (and not parvocellular) division of the PVN, a finding consistent with stimulation of magnocellular neurosecretory processes. Furthermore, under the above conditions of magnocellular hyperactivity, high levels of 11C-CCK receptor binding were observed in all the accessory magnocellular nuclei. Autoradiographical techniques carried out on the SCN demonstrated that the increase in 11C-CCK binding in the saltloaded rat reflected a 3X increase in Bmax, with no change in Kd. In conclusion, CCK receptor binding site density in the PVN and SCN appeared to be modulated with changes in magnocellular activity, this may reflect a role for CCK in neurosecretory and neuroendocrine release processes.


Cholecystokinin octapeptide (CCK-8) is one of several brain-gut peptides. In the CNS it is found in high concentrations in the cortex and fulfills many of the criteria expected for neurotransmitters. In the pancreas, it stimulates the release of amylase, presumably by increasing the hydrolysis of polyphosphoinositides which mobilizes intracellular calcium levels. Because the biochemical mechanism of action in the brain is poorly understood and because of the lack of specific antagonists against the central type of CCK receptors, we have developed a monoclonal antibody (LTD14.JR) against this peptide. Mice were immunized with CCK-8 conjugated to keyhole limpet hemocyanin and antibodies were screened by RIA. Spleen cells from mouse with high affinity antibody were fused with mouse myeloma. Hybridoma cultures were screened for antibody activity using ELISA. LTD14.JR was identified as the candidate for culturing and ascites fluids were produced in mice. RIA determinations revealed that the purified LTD14.JR recognized the L-terminal fragments of CCK-8 with nM affinities, while N-terminal fragments of CCK-8 were inactive in the uM range. In radioligand binding assays, LTD14.JR decreased the affinity of CCK-8 by 10 fold in both the cerebral cortex and pancreas; in pancreatic acinar preparations, LTD14.JR inhibited the response of CCK-8 in the amylase and PI assays. These results indicate that LTD14.JR behaves as a CCK antagonist which should prove useful in assessing the physiological significance of CCK in the CNS.

424.11 REDUCTION OF CCK-8 BINDING IN THE NUCLEUS OF THE SOLITARY TRACT IN UNILATERALLY NODESECTIONED RATS. E.E. Ladenheim, E.C. Smith and R.C. Witter. Dept. of VSMC, College of Veterinary Medicine, Washington State University, Pullman, WA 99164 and WO Regional Program in Veterinary Medical Education. University of Idaho, Moscow, ID 83843.

High affinity binding sites for cholecystokinin (CCK) have been identified in the nucleus of the solitary tract (NOS), postrema (AP) and the spinal trigeminal complex (SP5) (Garth et al., 1983; Moran et al., 1986). Previous work from our laboratory has shown that pretreatment of rats with the neurotoxin capsaicin produces a significant depletion of CCK receptor binding in the NST and AP but not in SP5 (Ladenheim et al., 1986). Since capsaicin destroys small diameter, unmyelinated sensory neurons, the receptors in the NST and AP may be located on capsaicin-sensitive vagal afferent terminals. In order to investigate whether capsaicin-induced depletion of CCK receptor binding may be attributed to the destruction of vagal afferent neurons, we examined high affinity binding of 125I Bolton-Hunter labeled CCK-8 in the brains of unilaterally nodosectomized rats. In confirmation of our previous report we found that capsaicin pretreatment decreases the density of CCK receptor binding in the medial and commissural NST and in the AP. In addition, we found that unilateral nodosectomy markedly reduces the density of binding in the medial and commissural NST on the side ipsilateral to the transected ganglia but produces no significant depletion in the AP. CCK receptor binding on the side contralateral to the lesion does not differ from that of controls. These results indicate that the receptors located in the NST and AP may be located on intact primary vagal afferent terminals. The fact that unilateral nodosectomy decreases CCK binding ipsilaterally in these regions suggests that the receptor loss in capsaicin-treated animals is due to the destruction of vagal sensory neurons. The failure of unilateral nodosectomy to diminish CCK binding in the AP suggests that either the AP receives significant capsaicin-sensitive innervation from the contralateral vagal nerve or that the CCK receptors in this region are not vagal dependent. These data are consistent with the possibility that central CCK receptors modulate the activity of vagal afferent nerves. Supported by NIH grants ROI NS21805 and ROI NS20561.

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For our studies of the structure-activity relationships of CCK-1-8 or CCK-26-33 for its receptors, we have synthesized metabolically and chemically stable analogs of the N-terminal fragment heptapeptide CCK-1-7 and hexapeptide, CCK-1-6. Previously the acetylated peptide analogs were found to inhibit the 0.3 nM CCK induced release of enzymes from guinea pig pancreatic acinar cells with potencies of 5-7 nM. The idea was to have affinities for central CCK receptors of guinea pig cortex in the range of 20 uM. Analogs with Nle substituted for Met in the peptide and with the carboxyl amide alkylated displayed greater potency in inhibiting CCK in guinea pig pancreatic secretion. Specifically, Ac Nle1, N2 CCK-2-6 dimethylamide and Ac Nle3,5 CCK-2-7 ethylamide displayed potencies of 0.18 uM and 0.034 uM, respectively (R.T. Jensen). The unsulfated peptides were much less potent. These are the most potent peptide inhibitors on guinea pig pancreas to date. We are preparing more alkylamide structures of the CCK 2-7 fragment and will determine potencies in pancreatic function and central receptor binding. More potent specific inhibitors of CCK may serve to delineate the central functions of the neuropeptide.
**424.13** ANTAGONISM OF CHOLECYSTOKININ-INDUCED EFFECTS ON NEURONS IN THE RAT NUCLEUS ACCUMBENS BY LORGLUMIDE. Richard J. Kasser, Xiu-Ti Hu, and Rex Y. Wang, Dept. of Psychiatry and Behavioral Science, SUNY Stony Brook, Stony Brook, NY 11794.

Makovec et al., (Arzneim.-Forsch. Drug Res. 36:98-102, 1986) reported that lorglumide (CR-1409, Rotta Labs, Monza, Italy), a new guanidino acid derivative, potently inhibits cholecystokinin (CCK) binding to receptors in rat brain and mouse cerebral cortex. Our lab has previously shown that iontophoresis of proguggide (0.1 M), a prototypic CCK antagonist, effectively blocks CCK-induced excitation in the nucleus accumbens (NAC). Whole cell recordings were performed under sodium pentobarbital anesthesia in freely moving rats. Although proguggide has a low affinity for CCK receptor sites, the present study was to test whether lorglumide is a potent and selective antagonist of CCK-induced excitation in rat NAC by using single unit recording and microiontophoretic techniques.

Rats were anesthetized with sodium pentobarbital and mounted in a stereotactic apparatus. NAC cells were recorded using standard electrophysiological techniques. As previously reported (White and Wang, 1984), many NAC cells were quiescent. Therefore, the effects of CCK and lorglumide were studied on glutamate (GLUT)-induced as well as spontaneous neuronal activity. Iontophoretically applied CCK activated a subpopulation of both quiescent and spontaneously active cells and, as previously described (Wang and Hu, Brain Res. 380:363-367, 1986), markedly potentiated the activity evoked by GLUT. Iontophoretically applied lorglumide (0.5 M) but not nacl (0.5 M) blocked CCK-induced activation (n=13) and CCK potentiation of GLUT-induced activation of NAC cells (n=15). Intravenous lorglumide (0.3 mg/kg) also effectively prevented the excitatory effects induced by CCK (n=8). Iontophoresis of 1 M lorglumide failed to block the excitatory effect evoked by GLUT or the inhibitory effects produced by DA (n=4) and neuropeptides such as neurotensin (n=6), substance P (n=4) and somatostatin (n=7) on NAC cells, suggesting that lorglumide has a low affinity for CCK receptor sites. In contrast, iontophoretically applied CCK-induced effects are selective. However, lortogludeum at high concentration (1 M), in addition to blocking CCK-induced effects, potently and selectively inhibited GLUT-induced excitation in 7 cells. Lorglumide never potentiated CCK-induced activity.

In conclusion, the main results of the present study indicate that lorglumide is at least 10,000 times more potent than proguggide in blocking CCK-induced effects on NAC cells. If the finding can be extended to other brain areas, lorglumide has the potential to be the CCK antagonist of choice for use in the CNS. (Supported by USPHS Grants MH-41440, 41696 and 00378 to RYW).

ANG receptors in the regulation of body fluid homeostasis. Angiotensin (ANG) is a circulating peptide involved in the peripheral regulation of water and salt metabolism and cardiovascular function. In addition, ANG has actions in the brain through stimulation of specific microencephalins. ANG receptors are located in the circumventricular organs, the subfornical organ (SPO) and in brain areas protected by the blood brain barrier. The central ANG system has been reported to be one important factor contributing to the DOCA-salt experimental model of hypertension. We therefore compared the concentration of ANG receptors in specific brain nuclei of control (C), DOCA treated (D), DOCA treated and salt deprived (DS) rats. ANG binding is measured using specific ANG radioisotopes.

The principal peptide responding to water deprivation is angiotensin II (ANG II). We have therefore investigated binding sites for ANG II in distinct rat brain nuclei. Male Sprague-Dawley rats (225-250g) were used in this study. The animals were kept in a temperature-controlled room (24 ± 1 °C) with a 12-hr on/12-hr off lighting schedule and housed in standard laboratory metal cages. Laboratory food and water were provided ad libitum to one set of animals (control group) whereas only food was provided to the second set of animals (treatment group). The treatment group of animals were deprived of water for a period of 5 days. All animals were killed by decapitation between 09.00 and 11.00 hr. The brains were rapidly dissected out, meninges removed, and the brain tissue immediately frozen at -70°C in isopentane on solid carbon dioxide. Receptors for ANG II in brain nuclei were quantified by incubating 16 µm brain sections with a saturating concentration of 3 nM [125I]ANG II followed by autoradiography with computerized microdensitometry and comparison with [125I]-standards. Binding densities in fmol/mg (mean ± S.E.M.) obtained in the various brain nuclei for control (C) and water deprived (D) were respectively: SPO, 171 ± 12 (C) and 171 ± 10 (D); paraventricular nucleus (PVN), 116 ± 12 (C) and 131 ± 11 (D); area postrema (AP), 140 ± 8 (C) and 136 ± 10 (D); nucleus of the solitary tract (NTS), 166 ± 6 (C) and 131 ± 9 (D) and OVLT, 185 ± 15 (C) and 210 ± 20 (D). ANG receptors were significantly increased in selective brain areas of DOCA-salt hypertensive rats. In those areas, binding densities, in fmol/mg protein were: median preoptic nucleus, 131 ± 9 (C) and 182 ± 6 (D); subfornical organ, 130 ± 8 (C) and 201 ± 8 (D); paraventricular nucleus, 78 ± 7 (C) and 156 ± 10 (D) (p<0.01); area postrema, 30 ± 3 (C) and 94 ± 10 (D) (p<0.05). Binding site density was not different in arcuate hypothalamic nuclei: 124 ± 14 (C) and 128 ± 18 (D). Salt treatment alone increased ANG binding in the area postrema and nucleus of the solitary tract. However, in the subfornical organ, paraventricular nucleus and nucleus of the solitary tract, binding sites for ANG receptors induced by DOCA-salt were significantly higher than those produced by salt alone.

Our data indicate that ANG receptors are altered in central areas related to blood pressure control and fluid homeostasis in DOCA-salt hypertensive rats, and may play a role in the pathophysiology of this experimental model of hypertension.


Bombesin (BN)-like peptides and receptors are discrete peptides that may regulate neuronal activity (Farb et al., J. Neurosci., 5: 429, 1985). While BN causes hypothermia, hyperglycemia, grooming, satiety and unknown. Here we investigated if BN-like peptides altered hormone secretion in rats its second messenger remains unknown. BN or the structurally related gastrin releasing peptide (GRP) have been duplicated in the regulation of body fluid homeostasis. ANG II followed by autoradiography with computerized microdensitometry and comparison with [125I]-standards. Binding densities in fmol/mg (mean ± S.E.M.) obtained in the various brain nuclei for control (C) and water deprived (D) were respectively: SPO, 171 ± 12 (C) and 171 ± 10 (D); paraventricular nucleus (PVN), 116 ± 12 (C) and 131 ± 11 (D); area postrema (AP), 140 ± 8 (C) and 136 ± 10 (D); nucleus of the solitary tract (NTS), 166 ± 6 (C) and 131 ± 9 (D) and OVLT, 185 ± 15 (C) and 210 ± 20 (D). ANG receptors were significantly increased in selective brain areas of DOCA-salt hypertensive rats. In those areas, binding densities, in fmol/mg protein were: median preoptic nucleus, 131 ± 9 (C) and 182 ± 6 (D); subfornical organ, 130 ± 8 (C) and 201 ± 8 (D); paraventricular nucleus, 78 ± 7 (C) and 156 ± 10 (D) (p<0.01); area postrema, 30 ± 3 (C) and 94 ± 10 (D) (p<0.05). Binding site density was not different in arcuate hypothalamic nuclei: 124 ± 14 (C) and 128 ± 18 (D). Salt treatment alone increased ANG binding in the area postrema and nucleus of the solitary tract. However, in the subfornical organ, paraventricular nucleus and nucleus of the solitary tract, binding sites for ANG receptors induced by DOCA-salt were significantly higher than those produced by salt alone.

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Peptide Receptors II. Friday PM.
425.1 HYPOTHALAMIC GnRH NEURON-PITUITARY GONADOTROPH FUNCTION THROUGHOUT POSTNATAL DEVELOPMENT IN THE GUINEA PIG: A COMPARISON WITH THE PRIMATE M. S. Hoffman, M. D. Greenberg, and D. B. Plant. Department of Psychology, University of Pittsburgh School of Medicine, and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261.

The protracted delay in the onset of sexual maturation in primates appears to be occasioned by interruption of intermittent hypothalamic GnRH secretion from shortly after birth until the time puberty is initiated. This prepubertal hiatus in GnRH release is not interrupted by the inhibitory action of sex steroids, since appropriate developmental pattern in GnRH secretion, as inferred from the time course of circulating LH levels, is maintained in the absence of gonadal feedback. The guinea pig's pituitary may exhibit an analogous mechanism for the restraint of the reproductive axis during prepubertal life has been proposed by Nass et al. (Endocrinology 115:220, 1984). These studies reported that ovine GnRH insufflation did not result in an immediate rise in gonadotropin secretion. Instead, circulating LH was maintained at concentrations comparable to those of intact controls until approximately 30 days of age. In contrast, the foregoing findings prompted the present study, which was designed to describe for the guinea pig the timing of the onset of the non-gonadal prepubertal growth spurt and the associated LH surge, as inferred by Nash et al. (Endocrinology 115:220, 1984). With this regard, OVX and received permanent cannulae in the third ventricle of guinea pigs. Supported by NIH grants HD 13254 and MH 18273.

425.2 SEASONAL PLASTICITY IN SYNTACTIC INPUT TO LUTEINIZING HORMONE-RELEASING HORMONE (LH-RH) NEURONS IN THE SHEEP. J. K. Jackson, J. E. L. Kissinger, and G. A. Blanken. Developmental Neurobiology and Forestry Department, University of Idaho, Moscow, Idaho 83844.

LH-RH neurons and their projections constitute the final common pathway of a neural pulse generator which controls episodic secretion of LH. Exposure to field conditions of lighting, temperature and seasonal hormones signals act upon the LH-RH pulse generator to coordinate an annual rhythm in reproduction. In reproductive studies in sheep (Lehman et al. J. Comp. Neurol. 196:19), we hypothesized that structural plasticity of LH-RH neurons or their synaptic inputs are key factors underlying seasonal changes in LH secretion. To test this we compared the synaptic inputs of LH-RH neurons in adult intact ewes and in ewes with a mid- cortical lesion of the brain, specifically during the mid-luteal phase of their estrous cycle (n=5), with those of ewes perfused during anestrus (n=5). Brain were fixed with 4% paraformaldehyde with 0.2% glutaraldehyde, and processed for EM immunocytochemistry as before (Silverman & Wiltkin, J. Histochem. Cytochem. 22:69). Sections were silver-gold intensified prior to osmication, dehydration, and flat embedding. Sixty-five preoptic LH-RH cells and 60 non-immunoreactive (non-IR) neurons from the same sections were analyzed (approx. 6 LH-RH and 6 non-IR cells per animal). The mean length and percentage of plastic membranes bearing synaptic modifications (either synaptic clefts or density) were calculated while LH-RH neurons in both breeding season and anestrous ewes were relatively undifferentiated compared to non-IR neurons. The mean percentage of small membrane bearing synaptic modifications in breeding season ewes (1.16 ± 0.81, 4583 μm membrane examined was more than twice as large in anestrous ewes (2.14 ± 0.37, 1881 μm membrane examined). The mean length of all synapses examined was significantly different between the breeding season (LH-RH cells: 0.29 ± 0.03; non-IR cells: 0.31 ± 0.03; anestrous: LH-RH cells: 0.17 ± 0.02; non-IR cells: 0.27 ± 0.02). LH-RH neurons in the preoptic area of ewes during the breeding season possess a significantly greater density of synaptic inputs than those of ewes during anestrous. Further experiments are needed to determine whether these changes in synaptic innervation are driven by photosensory and/or hormonal signals. (Supported by NIH HD 1835 (NPR) and HD 18634 and HD 16655 (AID))


Intraventricular (iv) administration of NPY readily stimulates LH release in rats, whereas NPY acts as a food intake suppressant in the rat. We hypothesized that NPY would also act as a food intake suppressant in a manner that would be antagonized by α2-adrenergic receptor function. (Supported by NIH DK 37273, HD 08634 and HD 13703).


Recently, the structure of the GnRHa encoding gene in rat androgen have been derived, and the corresponding peptide sequences are now of interest for determining the role of different fragments of the peptide. Moreover, immunohistochemistry with a specific antisera to proGnRH has been successfully performed in the hypothalamus of the rat. AK-2 antisera was generated against synthetic proGnRH in the cell body in the macaque hypothalamus (Ronnekleiv et al., Neuroendocrinology 46(1), 1987). Presently, we are reporting on the localization of proGnRH in the rat hypothalamus in the hypothalamic region. While LHRH neurons in both breeding season and anestrous ewes are relatively undifferentiated compared to non-IR neurons, the mean percentage of LH-RH membrane bearing synaptic modifications in breeding season ewes (1.16 ± 0.81, 4583 μm membrane examined was more than twice as large in anestrous ewes (2.14 ± 0.37, 1881 μm membrane examined). In control rats, LH levels were elevated for 60 min. This inhibition was significant (P<0.05; two-tailed test). The difference reflected an increased in synaptic density of both axosomatic and axodendritic synapses in the hypothalamic-pituitary-adrenal axis rather than operation of a specific pubertal control system. However the case may be, the present study fail to provide evidence for the view that the differentiation of cells in the hypothalamus is correlated in primates and guinea pigs. Supported by NIH grants HD 13254 and MH 18273.


The current results of the C121203 genes encoding human and rat proGnRH have been derived, and the corresponding peptide sequences are now of interest for determining the role of different fragments of the peptide. Moreover, immunohistochemistry with a specific antisera to proGnRH has been successfully performed in the hypothalamus of the rat. AK-2 antisera was generated against synthetic proGnRH in the cell body in the macaque hypothalamus (Ronnekleiv et al., Neuroendocrinology 46(1), 1987). Presently, we are reporting on the localization of proGnRH in the rat hypothalamus in the hypothalamic region. While LHRH neurons in both breeding season and anestrous ewes are relatively undifferentiated compared to non-IR neurons, the mean percentage of LH-RH membrane bearing synaptic modifications in breeding season ewes (1.16 ± 0.81, 4583 μm membrane examined was more than twice as large in anestrous ewes (2.14 ± 0.37, 1881 μm membrane examined). In control rats, LH levels were elevated for 60 min. This inhibition was significant (P<0.05; two-tailed test). The difference reflected an increased in synaptic density of both axosomatic and axodendritic synapses in the hypothalamic-pituitary-adrenal axis rather than operation of a specific pubertal control system. However the case may be, the present study fail to provide evidence for the view that the differentiation of cells in the hypothalamus is correlated in primates and guinea pigs. Supported by NIH grants HD 13254 and MH 18273.

In the last few years, it has become evident that the neurotransmitter y-aminobutyric acid (GABA) may have an important role in the control of gonadotropin secretion. However, the precise role played by the GABA receptors in the control of the gonadotropins has not yet been fully clarified. The main purpose of this study was to investigate the involvement of different GABA receptors on LH release and LH secretion in vitro and in vivo. In order to explore this issue we performed both in vitro and in vivo experiments using adult male intact Sprague-Dawley rats. In the in vitro experiments, a hypothalamic fragment consisting of the intact arcuate nucleus-medial eminence region (ARC-ME) was dissected and incubated in a Krebs buffer. LH release was evaluated during a 30 min incubation period. Incubation of the tissue with different concentrations of muscimol (10^{-6} to 10^{-4} M), a specific GABA-A receptor agonist, increased the release of LH from the ARC-ME fragment. Baclofen (10^{-4} M), a GABA-B receptor agonist, did not affect basal LH release. The effects of muscimol were completely blocked by the specific GABA receptor antagonist, bicuculline. In the in vivo experiments, the animals were implanted with a silastic cannula in the right jugular vein in order to collect frequent blood samples (every 10 min up to 80 min) before and after drug administration. In these experiments, the intravenous (iv) administration of aminoxyacetic acid (ADA, 25 mg/kg BW), an inhibitor of GABA catalysis which has been shown to increase brain GABA levels, completely inhibited the rise in LH induced by naloxone (5 mg/kg BW, iv). Baclofen (6 mg/kg BW, iv) injected 20 min before naloxone prevented the naloxone-stimulated LH release. On the other hand, muscimol was ineffective against the naloxone-stimulated LH release, while muscimol had an additive effect with naloxone to increase LH release from the ARC-ME. The results of this study suggest that the GABAergic system exerts both an inhibitory and stimulatory effect on LH secretion. The data indicate that the GABA-A receptors are responsible for the inhibitory effect observed in vitro and in vivo, particularly during situations of stimulated LH release and increased LH secretion.

In the next experiments, the specific GABA-A receptor antagonists were defined and their effects on LH release were determined. The finding that different GABA receptor types mediate opposite effects on LH release and LH may help to clarify the exact role of this neurotransmitter in the regulation of gonadotropin secretion.
425.9 IN VIVO LHRH AND 5-HIAA LEVELS AS MEASURED WITH PUSH-PULL PERFUSION OF THE ANTERIOR PITUITARY (AP) OF OVARIECTOMIZED FEMALE RATS WITH OR WITHOUT AN ESTROGEN IMPLANT. M. A. Barlow, G. A. Baber, and T. J. Ramirez (SPONSOR: L. Barr). Department of Psychology and Biophysics, University of Illinois, Urbana, IL 61801.

Herein, we report in vivo LHMM and 5-HIAA levels as measured with push-pull perfusion of the AP of ovariectomized (OVX) rats under the following conditions: 1) short term OVX (≤10 days), 2) long term OVX (>60 d), and 3) long term OVX with an estrogen implant. Rats (240-250 g) were anesthetized with Ketamine–Diazepam (1:10) for surgery. Bilateral ovariectomy was performed and a push-pull cannula was implanted aimed at the AP. After surgery, rats were kept in an individual cage at 22°C under 12L:12D photoperiod (lights on at 0500 h). Pituitaries were perfused at a flow rate of 11-12 µl/min with a modified KRP medium (containing 0.1 mM Bacitracin). Perfusate samples were collected on ice every 10 min, from 1100 to 1800 h. While being perfused, rats showed normal behaviors such as sleeping and grooming. An aliquot (20 µl) of each sample was immediately injected to an HPLC-EC for the analysis of 5-HIAA while the remaining of the sample was acidified and kept frozen until LHRH RIA. The individual data for monolayer cultures of enzymatically dissociated anterior pituitary gland. Supported by the Mellon Foundation.

These data suggest that LHRIF is a physiologically significant neurotransmitter simultaneously, 2) LHRH activity as measured with push-pull perfusion of the pituitary did not change as a function of those conditions. Long term OVX levels of 3,965.8 ± 611.5 pg/10 min were significantly lower (P < 0.05) than those observed in the group of short term OVX (60.0 ± 41.0 vs 5.24 ± 4.24 pg/min). These elevated levels of 5-HIAA were reduced to 671.4 ± 118.7 pg/10 min in the group of long term OVX rats stimulated with estrogen.

Results show that 1) push-pull perfusion of the pituitary can be used to analyze changes in neuropeptides and neurotransmitters simultaneously, 2) LHRH activity as measured with push-pull perfusion of the pituitary gland. Supported by HD-21588 and MH-37877.

425.10 PULSATILE GnRH RELEASE FROM THE HUMAN MEDIOBASAL HYPOTHALAMUS (MBH): IN VITRO SUPPRESSION BY MORPHINE. D.D. Rasmussen, M. A. Baber, and T. J. Ramirez (SPONSOR: L. Barr). Department of Psychology and Biophysics, University of Illinois, Urbana, IL 61801.

Neither hypothalamic pulsatile GnRH secretion nor the site of the putative GnRH pulse generating mechanism have been demonstrated in the human. Accordingly, an in vitro perfusion system was developed using human MBH slices (4 mm thick) from fetal (21-23 wk gestation) and adult hypothalamus (obtained at autopsy within 5.5 h of fetal death and 12 h of adult death). After a 3 h stabilization period, the slices were superfused with balanced salt solution containing 0.1 mM Bacitracin. Samples were collected on ice every 10 min, from 1100 to 1800 h. While being perfused, rats showed normal behaviors such as sleeping and grooming. An aliquot (20 µl) of each sample was immediately injected to an HPLC-EC for the analysis of 5-HIAA while the remaining of the sample was acidified and kept frozen until LHRH RIA. The individual data for monolayer cultures of enzymatically dissociated anterior pituitary gland. Supported by the Mellon Foundation.

425.11 BIOLOGICAL PROPERTIES OF LUTEINIZING HORMONE RELEASE INHIBITING FACTOR. Long-Chin Ahn, Jen E. Levine and Marc E. Freeman. Department of Biological Science, Florida State University, Tallahassee, FL 32306 and Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60201.

We have recently reported that the rat hypothalamus contains an inhibitor of release (Hahn and Freeman Endocrinology 120:483, 1987). This inhibitor, designated luteinizing hormone releasing hormone inhibitory factor (LHRIF), depressed LH-stimulated GnRH release in proestrus rats as well as in the olfactory cultures of enzymatically dissociated anterior pituitary cells (AP). The factor also depressed LH release induced by D-Ala<sub>2</sub>-Leu<sub>7</sub>-GnRH suggesting that LHRIF is not merely a peptidase. LHRIF had neither an inhibitory nor stimulatory effect on basal LH or prolactin secretion. Fraction P<sub>2</sub>—P<sub>3</sub> strongly inhibited the release of LH from the pituitary gland. Supported by the Mellon Foundation.

These data suggest that LHRIF is a physiologically significant neurotransmitter simultaneously, 2) LHRH activity as measured with push-pull perfusion of the pituitary did not change as a function of those conditions. Long term OVX levels of 3,965.8 ± 611.5 pg/10 min were significantly lower (P < 0.05) than those observed in the group of short term OVX (60.0 ± 41.0 vs 5.24 ± 4.24 pg/min). These elevated levels of 5-HIAA were reduced to 671.4 ± 118.7 pg/10 min in the group of long term OVX rats stimulated with estrogen.

Results show that 1) push-pull perfusion of the pituitary can be used to analyze changes in neuropeptides and neurotransmitters simultaneously, 2) LHRH activity as measured with push-pull perfusion of the pituitary gland. Supported by HD-21588 and MH-37877.


Several lines of evidence suggest that the arcuate nucleus (ARC) may generate neural signals for the release of LH in the male rat. These signals may be transmitted to GnRH neurons in the ME and/or ovary. In this respect, the effects of damage to ARC neurons could not be dissociated from those due to destruction of LH neurons in the ventromedial nucleus. In this respect, these lesions were classified as having either minimal or extensive damage to ARC. In the latter group, the vast majority of ARC neurons was destroyed. In the former group, the lesions did not involve the entire ARC. In both cases, the effects of damage to ARC neurons could not be dissociated from those due to destruction of LH fibers projecting to the ME. This study was designed to distinguish between these two possibilities by making arcuate lesions in ARC. All surgeries were performed on anesthetized male rats (100 g each), 5 days after surgery.

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Previous work indicated that naltrexone (NALT) is an opioid antagonist, increased serum LH concentrations only during the lutal phase of the estrous cycle in the sexually mature pig (Burb et al., Biol. Reprod. 55:1162, 1996). However, in the intact or ovariectomized (OVX) progesterone (P) treated prepuberal pig, NALT failed to alter LH levels (Burb, Reprod. 34:115, 1986). The following study was conducted to determine if development of endogenous opioid peptide (EOP) modulation of LH secretion in the prepuberal pig is mediated by a genetic or ontogenetic (E) prorising or is a brain maturation process independent of the ovary. Fifteen OVX pigs, 280 days of age and 112 ± 2.3 kg body weight (BW), received P (0.55 mg/kg BW) twice daily for 10 days. Pigs were divided into four experimental groups: saline treated controls (SALT), P treated saline treated controls (SALT-P), P treated OVX (OVX-P) which was prior to puberty and were classified as chronologically mature (CM) based on their age at the time of treatment. The remaining five pigs had displayed two or more estrous cycles prior to OVX (puberty = 197 ± 10 days), and were classified as sexually mature (SM). In addition, 5 pigs were OVX at 100 days of age and 77.9 ± 3.2 kg BW and were prepuberal (P) at the time of the experiment at 170 days of age. SALT and SALT-P pigs were OVX at 100 days of age and 77.9 ± 3.2 kg BW and were prepuberal (P) at the time of the experiment at 170 days of age. All SALT-P and SALT pigs were OVX at 100 days of age and 77.9 ± 3.2 kg BW and were prepuberal (P) at the time of the experiment at 170 days of age.

425.14 BINDING OF RADIOIODINATED FSH-RELEASING PROTEIN (FRP) TO PITUITARY MEMBRANES AND LEUKEMIA CELLS. Carolyn A. Capanza and Wylie Vale (SPON: R. Evans). The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037.

Recently, several proteins regulating cell secretion, division or differentiation were found to share significant sequence homology. These proteins include Transforming Growth Factor (TGF-β), Inhibin, FSH-Releasing Protein (FRP), Melanin Inhibiting Protein (MIP), and Pigment Developing Factor (PDF-C). Inhibin is a heterodimeric protein composed of inhibin α subunits (αA, αB), both initially purified from gonad extracts. The first biological activity associated with FRP was the ability to maintain the biosynthesis and release of FSH from rat pituitary cells in vitro. Because receptor binding is assumed to be the first step in FRP action, it was of interest to characterize the binding of FRP to pituitary cells. FRP was radiolabeled using chloramine T and purified by HPLC. Using a crude rat pituitary membrane preparation, only 3.5% of the total counts added bound, and approximately 50% of these counts could be displaced using a preparation of native FRP. Because the heterogeneity of cell types within the pituitary may contribute to the low amount of binding, it was of interest to look for other sources of receptor for initial characterization.

Recently, Eto and coworkers described the isolation and characterization of Erythropoietin Differentiation Factor (EDF) which stimulates the differentiation of mouse erythroleukemia cells (MEL cells). EDF is also a homodimeric protein that appears to be identical to FRP based on the identity of the N-terminal sequence of both proteins. Because EDF/FRP is biologically active on MEL cells, the binding of 125I-FRP on MEL cells was explored. Significantly higher specific binding was observed using MEL cells as compared to pituitary membranes. Incubation of 5 x 10^6 MEL cells with 125I-FRP (0.15 nM) resulted in the binding of 10-15% of the total counts added, about equal to the highest binding of native FRP as unlabeled competitor. The estimated Kd of the MEL cell FRP binding site is 0.3 ± 0.4 nM. Unrelated proteins, FSH and EGF, did not cause displacement of the 125I-FRP from MEL cells. Other methods of binding characterization were also explored. 125I-FRP also binds to monocyte leukemia cells suggesting that FRP binding may be common to hematopoietic cell types in addition to specific cell types in the pituitary. Further characterization of the pituitary and the MEL cell FRP binding sites may illuminate the physiological relevance of FRP.


Administration of MSG to neonatal rats produces lesions in the arcuate nucleus (ARC) of the hypothalamus including the tuberoinfundibular dopamine cells (TIDA) which tonically inhibit prolactin secretion. The present study (1) examined if MSG administration (Burb et al., Biol. Reprod. 34:115, 1986) effects arcuate nucleus (ARC) morphology and/or TH-immunoreactivity. The present study (1) examined if increased TH staining in the ARC was present in MSG-treated rats. In the MSG-treated pigs, increased TH staining was observed in the TIDA cell group, which is located in the periaqueductal gray. These results are consistent with the finding that MSG treatment results in a decrease in prolactin levels in the MSG-treated pigs. This decrease in prolactin secretion in the MSG-treated pigs is mediated by a decrease in TIDA cell function.

425.16 THE ROLE OF INCREASED CORTISOL SECRETION IN THE SUPPRESSION OF GnRH PULSE GENERATOR ACTIVITY IN MONKEYS PLACED ON A REGIMEN OF REDUCED FOOD INTAKE. J.T. Catterall, Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109, and S.W. Carlson, Department of Chemistry, Eastern Michigan University, Ypsilanti, MI 48197. Supported by NIH grants HD20789 and HD20887.

Previous experiments have demonstrated that 50-75% of adult male monkeys placed on a reduced food intake (RFI) regimen (e.g., consuming about one-third of their normal 2220 kcal food intake for approximately 3 weeks) have a suppression of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion resulting from reduced gonadotropin releasing hormone (GnRH) secretion (McEwen, B.S., Endocrinology 118: 182-225, 1986). The decrease in gonadotropin secretion during RFI is accompanied by an increase in circulating levels of cortisol. To examine this relationship more closely, 6 castrate adult male rhesus monkeys were placed on the RFI regimen and blood samples were collected by femoral venipuncture every 2-3 days for measurement of LH, FSH and cortisol. Results showed that circulating cortisol levels rose sharply (to levels between 80-120 ug/dl), coincident with the decline in circulating LH and FSH secretion, in monkeys which had a decrease in gonadotropin secretion during RFI. In contrast, no abrupt increase in circulating cortisol was detected in monkeys which did not show a decrease in circulating gonadotropin levels during RFI. This correlation suggested that a rise in circulating cortisol may play a role in suppressing gonadotropin secretion during RFI. To test this hypothesis, 5 additional monkeys were given daily injections (i.m.) of hydrocortisone acetate (HCA) kindly donated by the Upjohn Co.) in a regimen designed to mimic the rise in cortisol found during RFI (i.e., increasing doses from 5-20 mg/kg over a 3-4 week period). Blood samples were collected 3-4 times per week by femoral venipuncture. All 5 monkeys showed a pattern of rising cortisol levels similar to the rise in serum cortisol found during RFI, including a peak level of 500-600 ng/ml. However, neither serum LH or FSH decreased in the 5 monkeys which received HCA. These results indicate that the rise in circulating cortisol is not responsible for the suppression of GnRH neuronal activity during RFI.
426.1 THE EFFECTS OF SURAL NERVE STIMULATION ON THE FIRING OF SOLEUS MOTOR UNITS IN MAN. C.G. Kukulka, D.A. Brown*. Physical Therapy Lab. The University of Iowa College of Medicine, Iowa City, IA 52242.

Cutaneous afferent effects on motorneuron excitability have been studied primarily using noxious skin stimulation to evoke flexion reflexes. Much less is known about the effects of nonnoxious cutaneous stimulation. The purpose of this study was to evaluate the effects of nonnoxious levels of electrical stimulation of the sural nerve on the discharging of human soleus motor units. Bipolar fine wire electrodes were used to record motor units intramuscularly from soleus muscle. Electrical stimuli were delivered to the sural nerve inferior to the lateral malleolus. Two stimulation paradigms were used. One consisted of single pulses at 3 pulses/s. The second consisted of 500/s trains of 0.1 msec pulses of durations varying from 5 to 10 msec delivered at 3 trains/s. The effect of stimulation was evaluated by constructing peristimulus time histograms. Two general findings were observed. The first, produced by pulse train stimulation, consisted of short latency excitation-inhibition-excitation, initiated at latencies of 42-44 msec and lasting 10-14 msec. The inhibitory effect has been consistently seen in all 3 subjects tested to date. The excitatory effects are more labile and appear highly sensitive to the intensity of stimulation. The second finding (Fig. B), produced by pulse train stimulation, consisted of long latency excitation-inhibition of much longer duration. Excitation was observed at latencies of 54-56 msec, and lasted for durations of 64-66 msec. Inhibition was observed at 120-124 msec and lasted for 26-30 msec. These findings suggest that the two different types of stimulation may activate different spinal and/or supraspinal pathways involved in nonnoxious cutaneous reflexes.

426.2 NONLINEAR IDENTIFICATION OF A HAMMERSTEIN MODEL OF HUMAN TRICEPS FLEXOR PM REFLEX FUNCTION. II. Norbert Bogdan, G. David. Biomedical Engineering Unit, McGill University, Montreal, Canada.

The dynamic relation between the angular velocity of a limb (input) and the resulting mean rectified electromyogram (EMG) (output), sometimes termed the stretch reflex, has been previously modeled as a half-wave rectifier (static nonlinear, N, element) followed by a linear filter (dynamic or L element). A more realistic nonlinear dynamic model is known as a Hammerstein system (see figure below). Until recently system identification techniques appropriate for Hammerstein systems required Gaussian-white inputs (i.e., Gaussian first-order amplitude density function and constant first-order power spectral density function) and a monotonically increasing (or decreasing) L element. These techniques have not been used because Caucasian-white angular displacement perturbations are difficult to arrange in practice.

Hunter and Korenberg (1986) have recently presented a Hammerstein identification technique which does not require either Gaussian-white noise or a monotonically N or L elements. Furthermore, the technique is nonparametric so a priori assumptions about the form of the nonlinearity (N) and order of the dynamic linearity (L) are not required.

The Hammerstein nonlinear system identification method involves an iterative procedure in which the static nonlinear component of the system is represented by a polynomial (whose order is determined by a model order selection criterion) and the dynamic linear element, L, is estimated using either singular value decomposition or Toeplitz matrix iteration techniques. We have found that the technique is robust in the presence of large amounts of noise corrupting the EMG signal.

Fitzhugh's large, non-Gaussian, non-white stochastic angular displacement perturbations of the human foot about a fixed mean ankle joint angle, we have successfully used the technique to obtain nonparametric estimates of the N and L elements of the Kukulka and Hunter binary reflex representation of stretch reflex dynamics (see Part II). Representative N and L elements determined by the method are shown in the figure. (Supported by NSERC and MRC)

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426.3 NONLINEAR IDENTIFICATION OF A HAMMERSTEIN MODEL OF HUMAN TRICEPS SURAE STRETCH REFLEXES. PART II: RESULTS. R.E. Kearney, J.V. Hunter, & P.L. Weiss, Biomedical Engineering Unit, McGill University, Montreal, Quebec, Canada.

Stretch reflexes in the human triceps surae may be described in terms of the dynamic relation between joint velocity and the resulting EMG activity. This relation is very nonlinear; we have previously modeled it as a Hammerstein system consisting of a static nonlinearity cascaded with linear dynamics [2]. The form of the static nonlinearity was found by trial and error to resemble a unidirectional rate sensitive element or half-wave rectifier. This model gave adequate descriptions of reflex behavior for particular conditions, but only incompletely since estimates for the linear dynamic component changed with displacement amplitude and joint position. One explanation for this behavior is that the static nonlinearity is more complex than a unidirectional rate sensitive element. We undertook to explore this possibility using a new nonlinear identification method which produces direct estimates of both the static nonlinear and the dynamic linear components of Hammerstein systems [1].

Subjects were trained to develop constant mean levels of ankle torque while subjected to perturbations of ankle angular position. The perturbation was a stochastic signal containing significant power to 50 Hz; the peak-to-peak amplitude of the perturbation was modulated from 0.0 to 0.25 rad in a ramp waveform with a period of 16 s. Ankle position, torque and EMG activity were sampled at 500 Hz for 80 s. Estimates of the static nonlinear and dynamic linear components of a Hammerstein model were then obtained using the iterative procedure described in [1].

The static nonlinearity and linear dynamics, represented by the impulse response function, were found to have characteristic forms which were similar from trial to trial and subject to subject. In agreement with our previous findings the linear impulse responses were dominated by a large excitatory peak at about 40 ms. The static nonlinearity displayed a definite direction dependent rate sensitivity: positive velocities (stretch of triceps surae) were passed with a much higher gain than negative velocities. The nonlinear models identified in this work are in general agreement with our previous findings. However, the new models predicted EMG responses more accurately than our previous models. Experiments are in progress to determine whether these results in models having a wider range of applicability.

Supported by the Canadian Medical Research Council and the Natural Sciences and Engineering Research Council of Canada.

426.5 INCREASED INHIBITORY EFFECTS ON CLOSE SYNERGISTS DURING MUSCLE FATIGUE. L. Hayward, D. Breitbach and W.G. Ryiner. Dept. of Physiology, Northwestern Univ., Chicago, IL 60611.

Investigations of human motor unit discharge during sustained maximum voluntary contractions have demonstrated that motor unit firing rate declines during muscle fatigue. This fatigue-induced change is presumed to help optimize force generation in the fatiguing muscle. The rate modulation may be attributed to central changes (Kennell & Monster, Exp. Brain Res. 46:191,1982), recent evidence supports the role of a peripheral feedback mechanism (J. Physiol. 379:451,1986). In this study we examined some characteristics of this fatigue related feedback mechanism by comparing the influence of MG fatigue on force production of a non-fatigued close synergist, SOL, in decerebrate cats.

MG was electrically fatigued by muscle-nerve stimulation at 1.3 x Group I threshold (xT) and the influence of fatigue-related afferent feedback was measured in the non-fatigued soleus muscle. Force, EMG and length were recorded from MG and SOL during either 3 sec (25-50Hz) stimulation of MG muscle-nerve or ramp stretches (3-8mm) of MG. Initial SOL force was established by crossed flexion of the ankle. A decrease in SOL force was recorded during isotonic or stretch stimulation of MG. This reflexly induced decrement in SOL force increased as MG force production increased. During fatigue (defined as MG force generation at 50% original value) this decrement in SOL force increased significantly greater (p<.05) after fatigue. After 30-50 minutes rest SOL force decrement during MG stimulation returned to control values.

This reflex inhibition may be mediated by afferents stimulated by mechanical, thermal or metabolic changes during fatigue. To identify the afferents responsible for the reflex inhibition of the SOL force during MG stimulation and fatigue, the MG muscle-nerve was sectioned and stimulated proximally. With stimulus intensities in Group I threshold range (1.1-1.5xT) low frequency stimulation resulted in no reflex or no effect on SOL force generation. However, higher stimulation frequencies (100-200 Hz) consistently produced small decrements in SOL force, suggesting a possible role for Group II afferents in the reflex inhibition. In SOL decrements were produced only at higher stimulus intensities, 20xT (50-200Hz), within the range of Group III and IV afferents. Since these afferents are sensitive to normal and chemical stimuli it is possible that their discharge increased during muscle fatigue, contributing to the increase in SOL inhibition reported here. This study supports the proposition that small fiber afferent discharge elicited during muscle fatigue inhibits regional MN pools.

426.6 PROLONGED MUSCLE STRETCH CAN MODIFY REFLEX AND VOLUNTARY MUSCLE ACTIVATIONS IN SPASTIC CEREBRAL PALSY. F. Malouin, F. Tremblay*, C.L. Richards and F. Dumas*. Physiotherapy Department, Faculty of Medicine, Laval University, Quebec City G1K 7P4.

We studied the short-term effects of a single session of prolonged muscle stretch (PMS) of the plantarflexors on reflex and voluntary muscle activations in 21 children (3 to 14 yrs) with spastic cerebral palsy (11 hemiplegics and 6 hemiplegics) assigned to an experimental (exp) and a control (ctl) group. Neurophysiological responses were measured with the child sitting (hip and knees flexed, with the right foot attached to a footplate. The ankle angle was aligned with the axis of the footplate and force production of the non-fatigued close synergist, SOL, in decerebrate cats. MG was electrically fatigued by muscle-nerve stimulation at 1.3 x Group I threshold (xT) and the influence of fatigue-related afferent feedback was measured in the non-fatigued soleus muscle. Force, EMG and length were recorded from MG and SOL during either 3 sec (25-50Hz) stimulation of MG muscle-nerve or ramp stretches (3-8mm) of MG. Initial SOL force was established by crossed flexion of the ankle. A decrease in SOL force was recorded during isotonic or stretch stimulation of MG. This reflexly induced decrement in SOL force increased as MG force production increased. During fatigue (defined as MG force generation at 50% original value) this decrement in SOL force increased significantly greater (p<.05) after fatigue. After 30-50 minutes rest SOL force decrement during MG stimulation returned to control values.

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The decerebrate cat has long been used as a model of rigidity, hypertonia, and spasticity. However, a consistent and reliable method for the quantitation of this hyperreflexive behavior has been lacking. We have developed a mechanographic device that provides a quick, precise, and objective measurement of a decerebrate cat's hindlimb, with the simultaneous recording of the reflexive force produced by the limb. These experiments were designed to determine the dose-effect curve of the action of the antispasmodic drugs, diazepam, baclofen, and chlorpromazine.

Cats were anesthetized with a mixture of halothane and nitrous oxide and tracheotomized. A bilateral carotid occlusion was performed. The animals were given saline and drug delivery. Following trephination of the skull, a mid-sagittal incision was made, and the brain was removed. The muscle length was established by crossed flexion of the ankle. The animals were attached to a footplate, and the ankle axis was aligned with the footplate. The ankle angle was aligned with the axis of the footplate and force production of the non-fatigued close synergist, SOL, in decerebrate cats. MG was electrically fatigued by muscle-nerve stimulation at 1.3 x Group I threshold (xT) and the influence of fatigue-related afferent feedback was measured in the non-fatigued soleus muscle. Force, EMG and length were recorded from MG and SOL during either 3 sec (25-50Hz) stimulation of MG muscle-nerve or ramp stretches (3-8mm) of MG. Initial SOL force was established by crossed flexion of the ankle. A decrease in SOL force was recorded during isotonic or stretch stimulation of MG. This reflexly induced decrement in SOL force increased as MG force production increased. During fatigue (defined as MG force generation at 50% original value) this decrement in SOL force increased significantly greater (p<.05) after fatigue. After 30-50 minutes rest SOL force decrement during MG stimulation returned to control values.

This reflex inhibition may be mediated by afferents stimulated by mechanical, thermal or metabolic changes during fatigue. To identify the afferents responsible for the reflex inhibition of the SOL force during MG stimulation and fatigue, the MG muscle-nerve was sectioned and stimulated proximally. With stimulus intensities in Group I threshold range (1.1-1.5xT) low frequency stimulation resulted in no reflex or no effect on SOL force generation. However, higher stimulation frequencies (100-200 Hz) consistently produced small decrements in SOL force, suggesting a possible role for Group II afferents in the reflex inhibition. In SOL decrements were produced only at higher stimulus intensities, 20xT (50-200Hz), within the range of Group III and IV afferents. Since these afferents are sensitive to normal and chemical stimuli it is possible that their discharge increased during muscle fatigue, contributing to the increase in SOL inhibition reported here. This study supports the proposition that small fiber afferent discharge elicited during muscle fatigue inhibits regional MN pools.


Three excitatory amino acid receptor subtypes have been identified in mammalian CNS. Classification of this hyperreflexive response was achieved through the use of specific agonists: N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and quisqualate (Quis). Pharmacological and functional characterization of Quis and KA receptors are incomplete, since no selective antagonists have been identified. However, 2-amino-7-phosphonohexanoic acid (AP7) and ketamine are potential selective competitive antagonist at the NMDA receptor. This is an attempt to determine the specificity of NMDA receptors with a pharmacological device that would be a potent and selective competitive antagonist at the NMDA receptor. This is an attempt to determine the specificity of NMDA receptors with a pharmacological device that would be a potent and selective competitive antagonist at the NMDA receptor. This is an attempt to determine the specificity of NMDA receptors with a pharmacological device that would be a potent and selective competitive antagonist at the NMDA receptor. This is an attempt to determine the specificity of NMDA receptors with a pharmacological device that would be a potent and selective competitive antagonist at the NMDA receptor.

Following a C1 section, monopelvic cats (2.0 to 4.0 kg) were artificially resired and halothane anesthesia was discontinued. A partial laminectomy was performed and dorsal and ventral spinal roots L6-S1 were isolated and cut. Supramaximal stimulation (10x threshold) was applied to a dorsal root and the resulting monosynaptic and polysynaptic reflexes were recorded from a ventral root. The amplified potentials were averaged and the areas under the curves integrated for analysis. Drugs were administered intravenously in saline. AP7 (3, 10 and 30 mg/kg), picrotoxin and selective decerebrate rigidity. This decrease was rapid (occurring as soon as 4 minutes after the 3 mg/kg dose) and was both time and dose dependent. However, there was no effect of AP7 on the polysynaptic reflex at each dose. The effect was short in duration, with the reflex area returning toward baseline levels within 20-30 min of dosing. Neither AP7 nor ketamine had any significant effect on the monosynaptic reflex at each dose. However, AP7 produced a significant decrease in the polysynaptic reflex at each dose. Polysynaptic reflexes result from activation of -motor neurons via interneurons in the spinal cord. The marked decrease in the polysynaptic reflex associated with AP7 and ketamine release a transmitter which acts at NMDA receptors. In contrast, the lack of effects on monosynaptic reflexes indicates that NMDA receptors probably not involved in the generation of the la afferent-motor neuron monosynaptic reflex (PSP). The preferential reduction of polysynaptic reflexes suggests that NMDA antagonists might reduce the hyperreflexia associated with spastic disorders without inhibiting the muscle weakness liability of current antispastic drugs. This research indicates that both competitive and functional NMDA antagonists have a potent and selective activity in the feline spinal cord.
426.9 ALTERATION OF A WITHDRAWAL RESPONSE IN RATS: DEPENDENCE OF DEVELOPMENT AND RETENTION ON SYMPATHETIC INNERVATION AND INHIBITORY ROLE OF CENTRAL SEROTONERGIC INPUT. M.F. ANDERSON and B.J. WINTERSON. Department of Physiology, University of New England College of Osteopathic Medicine, Biddeford, Maine 04005.

A variety of clinical conditions appear to involve hyperactive reflex loops between spinal cord segments and peripheral structures. These disorders include causalgia, Raynaud's disease, and chronic muscle spasm (somatic dysfunction). All of these disorders appear to have a sympathetic component and properties in common with animal models of reflex alteration. In the present study, the spinal fixation model, an example of reflex alteration, was used to assess the role of sympathetic activity in the generation and maintenance of a long term hindlimb asymmetry. Electrical stimulation (1.75-7 mA, 7 m sec, 100 Hz for 60 min) of rat hindlimb induced a maintained flexion which was quantified by applying 0.5 g weights until the stimulated and contralateral leg lengths were equal. Each of the induced flexion was lost by 72 h (“masked”) and a significant amount recovered subsequent to spinal transaction at 72 h (‘unmasked’).

Since serotonin has been implicated in the modulation of preganglionic sympathetic neurones, para-chlorophenylalanine (PCPA) was used to deplete serotonin stores. Following stimulation, control animals exhibited 24.9 g flexion. At 72 h, controls retained 6.0 g flexion which increased to 12.4 g after transsection. In an experimental group, administration of 0.01 mg of propranolol into the jugular sinus decreased post transsection flexion from 14.8 g to 6.3 g in ten min. Sympathetically (21-30 mg/kg/day guanethidine i.p. for six weeks) resulted in a decrease in post stimulation flexion (14.0 g) in comparison to controls (24.9 g). At 72 h, these animals retained 7.6 g and this was unaffected by transsection. Administration of PCPA (300 mg/kg i.p.) three days prior to fixation resulted in 25.9 g post stimulation, similar to controls (24.9 g). At 72 h, 10.9 g remained and was unchanged by transsection. Administration of propranolol as described above prompted a decrease in flexion to 5.3 g. A second group of PCPA treated rats was perfused with propanolol (0.01 mg/ml) for the duration of stimulation. Afterward, flexion (13.0 g) was markedly decreased compared to controls (24.9 g). At 72 h, 8.3 g of flexion remained and was unchanged by transsection.

These results suggest that: 1) Sym pathetic innervation is responsible in part for development of long term spinal memory. This was demonstrated by a decrease in flexion (12.4 g) developed in sympathectomized rats. 2) A adrenergic mechanism is implicated, as propranolol given either during stimulation or after transsection decreased flexion. 3) Masking is due to an inhibitory descending serotonergic influence, since pretreatment with PCPA abolished masking. (M.F. Anderson was supported by a Burroughs Wellcome Osteopathic Research Fellowship).


The experiments were designed to investigate what effects catecholamine fibers have on spinal reflex functions. Specific reference to electrophysiological parameters and correlates of hindlimb reflexes in adult rats. The goal was to design a simple, reliable, and relatively non-invasive test to measure the central excitatory state of animals. Since catecholamine fibers to hindlimb reflexes in adult rats. The parameters and correlates of hindlimb reflexes are useful in this respect because they are particularly dependent on CES.

Fourteen week old animals received an intrathecally injection of 250 μg of 6-OHDA in 20ul of ascorbic acid saline. Lesioned animals and age matched controls were tested 2 months post lesion. Reflex testing was done 1 hr. after performing a spinal transaction (at 1HP) under metophane anesthesia and in separate animals 3 days post transaction (at 3DP). The reflex test consisted of a series of graded electrical stimuli applied subcutaneously to the dorsum of the foot while EMG activity was recorded from the iliospinalis biceps femoris muscle. Testing was done before and after administration of L-DOPA or Clonidine.

Without administration of drugs, reflex evoked EMG activity consisted of low threshold, short latency and higher threshold, long latency components at 3DP, in control and lesioned animals. At 1HP only the low threshold, short latency component can be elicited. Stimulus response curves (SRC) were plotted for control and lesioned animals and were similar in both groups at 3DP. In the controls at 3DP, Clonidine and L-DOPA had similar effects on the SRC of the long latency component. After administration of L-DOPA or Clonidine at 1HP in controls, the long latency component could be evoked and had similar SRC's. In lesioned animals at HP and 3DP, L-DOPA showed no significant effect on reflex evoked activity, while Clonidine had effects similar to those seen in the controls.

Conclusions: Reflexly evoked EMG activity provides a relatively non-invasive test of the state of the spinal interneuronal circuitry. Results from reflex tests of 6-OHDA lesioned and control animals, before and after L-DOPA or Clonidine injections confirm previous reported effects of catecholamine fibers on hindlimb reflexes (E. Jankowska et al., Acts P. Scand. 70:369-386, 1967). The reflex test reported here will prove useful in assessing functional recovery from spinal cord injury.

426.11 THE PINEAL GLAND MODULATES BEHAVIORAL RESPONSE DIRECTION IN RATS. K.D. Dawson, D.P. Crowne* and A. Adelstein*. Psychology Department, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

The pineal gland is a median brain structure that may modulate behavioral responses to diverse stimuli. The pineal gland's role in environmental information provided by lateralized mechanisms in the central nervous system. Spontaneous turning behavior and orientation of stimulus-unilateral and bilateral visual and auditory stimulation were examined in each of 60 male Long Evans rats for one week preoperatively and for 4 weeks following one of three treatments: 1. anesthetization and scalp incision (AI control); 2. treatment 1 plus skull defect and electrode insertion (sham lesion); 3. treatment 2 plus electrolytic lesion (1 mA, 20 sec) of the pineal gland (pineal lesion).

Half of the animals in each treatment group were also subjected to postoperative darkness for 48 hours and subsequently returned to the standard LD 12:12 colony room. Each animal was tested twice per day during the light phase of LD 12:12, one test between 0500 and 1300 (morning), the other between 1500 and 2000 (evening).

Performance change ratios were computed that reflect ed each postoperative behavioral frequency relative to the corresponding preoperative frequency. Repeated measures analysis of variance on these scores showed animals with pineal lesion to be significantly (p<.05) different from sham and AI controls in exhibiting 37.5% loss of rightward orienting ability to bilateral visual stimulation and sighted responses. The results support the idea that the pineal gland modulate the excitation stimulation (37.5% loss of rightward orienting ability to bilateral visual stimulation and sighted responses. The results support the idea that the pineal gland modulate the excitation of the state of the spinal interneuronal circuitry. Results from reflex tests of 6-OHDA lesioned and control animals, before and after L-DOPA or Clonidine injections confirm previous reported effects of catecholamine fibers on hindlimb reflexes (E. Jankowska et al., Acts P. Scand. 70:369-386, 1967). The reflex test reported here will prove useful in assessing functional recovery from spinal cord injury.

426.12 WITHDRAWN
427.1 THE SENSITIVE PERIOD FOR THE EFFECTS OF MONOCULAR DEPRIVATION ON THE STARTLE RESPONSE AND THE PREPULSE INHIBITION OF ACOUSTIC STARTLE. Kevin P. Nance* and Hugh A. Tilson. Supported by grants from the NSF, NR 1 and the Deutsche Forschungsgemeinschaft (81/245/3). The unconditioned startle response is usually elicited by tactile or acoustic stimulation, and has been increasingly used to study behavioral effects of serotoninergic drugs. The present study examined the effects of several 5-HT agonists, quipazine, 5-Methoxy-N,N-dimethyltryptamine (5-MT) and 8-0H-DPAT, in male Fischer 344 rats in the mediation of the acoustic startle reflex.

427.2 VISUAL SYSTEM: DEVELOPMENT AND PLASTICITY. Frank Schaeffel* and Howard C. Howland. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853. Visual deprivation due to visual field restriction is growing in chickens, 3 days during the first 7 days. Immediately following the deprivation period the animals were perfused, the brains were cut and the cell size of neurons of the nucleus rotundus and ectostriatum and the volume of the rotundus were estimated from midsagittal sections. The results show, that monocular deprivation markedly affects the cell size in both areas if the treatment starts at 1 or 10 days posthatch. The differences between deprived and non-deprived eyes were significant, the volume of the nucleus rotundus parallel the results of the cell size measurements, whereby the second increase is delayed for 4 days, as severe effects as very early monocular closure. In contrast, deprivation onset at day 50 or later does not lead to detectable effects of the volume of the nucleus rotundus. The data of the present study suggest that visual deprivation is very efficient in producing axial myopia (increased depth of the vitreous chamber). Apparent evidence supporting this change in eye growth is the change in accommodation tonus in the deprived eye because myopia can also develop after the optic nerve has been cut (Trolin et al, Soc. Neurosci. Abstr. 18:77, 1992). However accommodation may still be important in regulating eye size and the retinal image quality, sinceaccomodative tonus in young chickens (n = 30eyes) by treating them with moderately powered, defocusing lenses for a period of 100 days. Lenses were attached to small leather hoods by velcro fasteners and were thereby easily removable for cleaning every 8-12 hours. It was found by infrared photoretinoscopy (Schaeffel et al, Vision Res. 26:1977, 1986) that chickens wearing lenses could keep images focused on their retina. The treatment resulted in a consistent shift in non-cycloplegic resting refractive state which was in the direction to compensate for the lenses. From measurements of transcorneal images in exposed eyes we conclude that the posterior nodal distance (PND) was increased when negative lenses were worn as compared to eyes over which positive lenses were worn, while the PND of eyes which developed is completely axial in origin the change in the PND can be compared to a continuous, progressive shift in refractive state. Calculations of the camera matches well with the changes observed in the living bird. However, the model predicts a change of only 50% (30-51%) than the one which could be expected if the full power of the applied lenses was compensated. From the model it follows that changes in eye growth are such that the scatter of the values of the refractive state was much smaller in deprived eyes. Supporting experiments, we believe that we triggered a regulatory mechanism for eye growth with the feedback mechanism closed. By contrast, deprivation experiments provide open loop conditions because compensatory eye growth cannot improve quality. Supported by DFG Sch 401/7-2 (F.-S.) and NIH EY-02994 (H.C.H.) and by a U.S. Dept. Agr. Res. Hatch Grant.

427.4 THE EFFECT OF ENucleATION ON TRANSSYNAPTIC TRANSPORT OF WGA-HRP IN A DEVELOPING RAT VISUAL PATHWAY. S. Itaya.

427.5 ACCESSORY OPTIC SYSTEM IN THE WALLABY SETONIX BRACHYURUS: EFFECTS OF EARLY UNILATERAL ENucleATION. L-A. Coleman* and L.D. Beazley.

427.6 EFFECTS OF MONOCULAR OCCLUSION ON LATERAL GENICULATE NUCLEUS (LGn) ANATOMY AND HISTOCHEMISTRY, AND ON RETINAL DOPAMINE SYSTEM IN INFANT Rhesus monkeys. M. Tippes, P. M. Iwane, J. Tippes, R. Fernandez* and J. A. Blackman.

427.7 Early unilateral enucleation results in an increased projection to primary visual centres ipsilateral to the remaining eye in the mature animal. At postnatal ages of 1-45 days (P1-45; P0 = day of birth). Center and Departments of Anatomy and Cell Biology, Pharmacology, and Ophthalmology, Emory University, Atlanta.

427.8 We also determined the effect of occlusion on the retinal dopamine system which has been implicated in visual information processing and in homeostatic regulation of retinal function. The dopamine biosynthesis, was reduced in the occluded eyes.

427.9 In sections reacted for the mitochondrial enzyme cytochrome oxidase, a marker sensitive to changes in neuronal metabolic activity, parvocellular layers in the LGn ipsilateral to the occluded eye were smaller compared to their counterparts connected to the open eye. Changes were greater in the LGn ipsilateral to the occluded eye. Supported by NIH grants EY05922, RR-05607, EY-03039.
Kitten-onset visual deprivations reduce the recordability of Y-cells in the lateral geniculate nucleus (LGN) of the cat. In contrast, adult-onset visual manipulations have a selective effect on X-cells. In the present experiments we have assessed the effects of duration of infant-onset deprivation, and therefore age of subject at the time of data collection, on the physiology and morphology of cells in the LGN. 22 cats underwent lid suture at between 3-4 weeks postnatal age. 12 were studied between 5 and 16 months of age, and 10 were studied between 17 and 29 months. In 18 of these same subjects we measured soma sizes as well, to permit direct comparisons of morphological and physiological effects of lid suture. These data were compared to those from 8 normal controls. During recording sessions one eye was immobilized by transecting cranial nerves III, IV, and VI. Animals were sedated, not anesthetized during recording sessions. Cells (representing the central St of visual space) in the lamina innervated by the paralyzed eye were classified as X or Y based on a standard battery of tests, those in laminae innervated by the mobile eye were classified solely on the basis of latency to optic chiasm stimulation.

Cats reared with lid suture and from which data were collected before 17 months of age showed a reduction in the encounter rate for Y-cells and a reduction in average cell body size in geniculate laminae innervated by the deprived eye. In contrast, no cat from which data were collected at or after 17 months of age showed a reduction in encounter rate for Y-cells, giving the appearance of a more normal X-Y-cell ratio. Similarly, the differences in average soma size between deprived and nondeprived laminae of the older subjects were smaller than those in the younger animals. Furthermore, both the physiological and morphological differences between nondeprived and deprived laminae are correlated (r=.58, p<.02). Preliminary observations suggest that these late changes in the effect of deprivation are not a recovery of Y-deprivations. In 19 of these same subjects we found a significant increase in GABA cell density. This difference, however, was not significant between normal and five adult cats enucleated unilaterally on embryonic days 44, 48, 50, 55 and 58. (Gestation is about 65 days.) Cells were classified as X or Y based on tests for linearity of spatial summation, receptive field size, spatial resolution and latency to optic chiasm stimulation.

In the enucleated animals, the vast majority of receptive fields were normally shaped, exhibited an on- or off-center organization and could be classified as X or Y type. Two significant differences between enucleated and normal animals were found, however. First, on the average, X cells in enucleated animals had larger receptive fields and lower spatial resolutions than normal. (A comparison of Y cells could not be made due to small samples.) Second, in animals monocularly enucleated before age 20, the ratio of X to Y cells was found. Analysis of the encounter rate for center frequency revealed that the change was due both to a gain of X cells and a loss of Y cells. These functional differences could reflect alterations in the arboreosis patterns of retinogeniculate afferents in the postnatally enucleated animals (Stryker et al., 1985). Such alterations might be eye removal creates a mismatch between the number of ganglion cells projecting to the LGN and the number of target neurons in the nucleus. [Supported by EY03991 from NEI, INT-8320440 from NSF and NINDS T32 NS07700.]
SAMPLE SIZE AND STATISTICAL POWER IN SOME MORPHOMETRIC PROBLEMS RELATED TO DEVELOPMENT AND AGING. N
Jeanpreta * G Leuba * K Krabfa * and J-M Fritschy * (SPON: J-P. Hornung) Institute of Anatomy, University of Lausanne, 1005 Lausanne, Switzerland.

In development and aging, changes in morphological parameters may go undetected due to inadequate sampling. The concept of power of a statistical test, associated with the type II error, may be used to avoid such erroneous conclusions. We have devised a program that allows computation of statistical power and appropriate sample size in the context of hierarchical analysis of variance. The problem is that of stagewise sampling when the number of individuals as well as the number of observations per individual to be determined. Several examples have been given based on existing data. To start with, the demonstration of statistical power for morphological changes considered to be of interest in development and aging, and, secondly, to illustrate a satisfactory strategy for the choice of sample size, both in terms of statistical validity of and economy. In an analysis of neuronal densities within a perpendicularly column of cortical tissue in human area 17 (Leuba, G. and Garey, L.J., Hum. Neurobiol., 6:11, 1987), it turned out to be more important to increase the number of individuals than that of observations per individual, in order to obtain greater precision and power. In an analysis of spine densities on given dendritic segments, in visual cortex of both mouse (Leuba, G., Neuropathol. appl. Neurobiol., 9:467, 1983) and human (Michel, A.E. and Garey, L.J., Hum. Neurobiol. 9:253, 1984), this was reversed, as a result of a study of the lengths of terminal dendritic segments in the lateral geniculate nucleus of the marmoset (Fritschy, J-M. and Garey, L.J., submitted). It seemed more important to increase the number of observations per individual than that of individuals.

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427.12


Intraventricular administration of 6-OHDA causes a modest decrease in visual cortical plasticity. The resulting depletion of norepinephrine (NE) cannot completely account for this change. Since the results are consistent with those reported by Fritschy and colleagues in the monkey (J. Neurosci. 6:266), Kasess and colleagues have reported that blocking cortical beta-adrenergic receptors results in a decrease in plasticity (Exp. Brain Res. 59:507). Because 6-OHDA treatment and blockade of beta receptors both cause supersensitivity of the beta adrenergic system in rats (J. Pharm. Exp. Ther., 203:125; J. Pharm. Exp. Ther., 207:446), we hypothesized that an increase in beta adrenergic receptor density might contribute to the decrease in plasticity. Therefore we examined the effect of 6-OHDA treatment on beta-adrenergic receptors in kitten visual cortex. Subjects were given vehicle solution alone (controls; n=6), a dose of 6-OHDA which depleted cortical NE and decreased visual cortical plasticity (4.8 mg; n=7), a dose of 6-OHDA which did not affect cortical NE and did not affect visual cortical plasticity (0.2 mg; n=7), or a dose of 6-OHDA which depleted cortical NE and decreased visual cortical plasticity (4.8 mg; n=7). Drugs were administered in either 4 or 6 single daily injections via intraventricular cannula. Animals were sacrificed 24 hrs after the last injection. Saturation assays were performed on homogenates of visual cortical tissue using [125I]pindolol (30-600nM). Icacinac (160nM) was used as a cold competitor to measure nonspecific binding. Radioactivity bound to tissue and retained on filters was measured. Scanned plates were generated using the ERASER processor (J. Pharm. Sci. 14:2129) for dissociation constant (Kd) and receptor density (Bmax) were determined in multiple assays for each animal.

Kd(*/-SEH) 111.0 (14.5) 107.9 (8.0) 96.2 (10.9) p<.05

Bmax(*/-SEH) 68.8 61.8 68.8 p<.05

Dose 6-OHDA treated 6-OHDA treated 6-OHDA treated

n=6 4 4

Kd 11.6 11.9 10.9 p<.05

Bmax 1404 1280 1120 p<.05

Cap surface area/CV 73.9 68.8 68.8 p<.05

Cap branches/µm 45.4 43.6 34.4 p<.05

Cap branches/mm 11.2 10.3 7.1 p<.05

Supported by NIH grants NS23310 to AJE and NRSA Short Term Research Training Program GM07405 to MCD.
427.15 CHANGES IN A GAP43-LIKE PROTEIN DURING DEVELOPMENT OF THE CAT VISUAL CORTEX. H. McIntosh, E. Mei, D. Parkinson, R. Villard & N. DeW. Department of Neurobiology, Washington University Medical School, St. Louis, MO 63110

The critical period in the development of the cat visual cortex extends from the time of eye opening to three months of age. During this time, both anatomical and physiological changes may occur as a result of altered sensory input or deprivation. We decided to test whether biochemical changes might also occur in this period. Our experiments focused on GAP-43, a growth-associated protein concentrated in growing axons that is closely related to the FI protein. Changes in phosphorylation of the FI protein have been observed in several laboratories during the critical period. This protein appears to be associated with the emergence of progressively more sophisticated feature-analyzing functions, thus performing an operation similar to 'principal component analysis,' an approach used in statistics for extracting 'interesting' structure (e.g., clustering) from complex input data.

One might therefore consider that COI is a candidate for a generic organizing principle for the emergence of feature-analyzing cells corresponding to observed functional areas, without requiring that the network be specifically instructed — either retrogradely or anterogradely — to form the connections, as a result of this organizing principle.

It is remarkable that the principle of constrained optimal inference induces the emergence of feature-analyzing cells corresponding to observed functional areas, without requiring that the network be specifically instructed — either retrogradely or anterogradely — to form the connections, as a result of this organizing principle.

I suggest that COI is a candidate for a generic organizing principle for perceptual development. The full exploration of the power and limitations of this approach, and its experimentally testable consequences, is in progress.

427.16 OPTIMAL TETANUS PARAMETERS FOR INDUCTION OF LTP IN RAT PRIMARY VISUAL CORTEX. B. Berry, T. J. Taylor, and H. Teyler* Department of Neurobiology, Northeastern Ohio University College of Medicine, Rootstown, OH 44272.

Tetanus-induced long-term potentiation (LTP) of evoked responses has been widely reported for the hippocampus. Hippocampal LTP is a strong candidate for a synaptic mechanism underlying the long-lasting increase in synaptic strength observed in the hippocampal LTP in memory, it is surprising that there are relatively few studies documenting the time course of LTP in areas which might be involved in memory and other forms of neural plasticity. One such brain area that is particularly interesting is area 17 (V1) of primary visual cortex. An enormous number of studies have focused on electrophysiological and neuroanatomical correlates of visual plasticity in area 17 of the cat. An interesting possibility is that cortical LTP might be an important mechanism in the development of visual areas outside the classical primary visual cortex.

Our results confirm this possibility. A number of studies have reported similar data for the developing rat visual cortex; however, these studies have emphasized the importance of sensory experience on the development of visual areas. Our results indicate that the development of visual areas outside the classical primary visual cortex is not dependent on sensory experience. This is consistent with the idea that cortical LTP might be an important mechanism in the development of visual areas outside the classical primary visual cortex.
427.19 A QUANTITATIVE STUDY OF RECEPTIVE FIELD PROPERTIES IN THE CAT'S SUPRASTYLAN VISUAL CORTEX AFTER NEONATAL LESIONS OF AREAS 17/18.

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Acute ablation of areas 17 and 18 in adult cats produce changes in the properties of neurons in the suprastylian visual cortex (Spear and Baumann, J. Neurophysiol., 1979; Guedes et al., Exp Brain Res. 1984). In neonatal kittens, lesions made during the first three weeks of life cause an increase in thalamic projections to the area PMLS, particularly from the lateral geniculate nucleus, but the properties of neurons in PMLS impaired so far appear to be unaffected, and this has been interpreted in terms of functional compensation during development (Tong et al., J. Neurophysiol., 1986).

We made lesions of areas 17 and 18 of 1-day-old kittens and recorded from units in PMLS after one year. For quantitative measurements, high-contrast drifting gratings or stationary, contrast-modulated gratings were presented on a display screen and spikes were collected under computer control to assess the selectivity for the direction, orientation, spatial frequency and temporal frequency of the stimulus. A separate analysis was performed for cells with peripheral receptive fields (centered at eccentricities > 20 deg) and those with receptive field centers that fell within 15 deg of the area centralis and whose entire extent lay within the area of visual field retinotopically equivalent to the lesion.

The ocular dominance distribution for cells in PMLS was normal, but the responsiveness of cells with receptive fields falling in the central visual field, corresponding to the lesion, was substantially reduced. However, 60% of cells with central receptive fields were direction selective and the distributions describing the variation in the strength of direction preference and the mean half-width of tuning were similar to those for normal adult PMLS. Tests with stationary, flashed gratings revealed that the proportion of orientation selective units amongst those cells with central receptive fields (36%) was only slightly reduced compared to unlesioned animals (45%). On the other hand, units with central receptive fields and unevenly limited spatial resolution (less than 1/deg) and a large proportion of them lacked low spatial-frequency attenuation ('low-pass' cells). On the other hand, some of the cells had peripheral receptive fields (outside the area corresponding to the lesion) had surprisingly high 'acuity'. Temporal selectivity appeared to be unaffected in the lesioned animals. We concluded that orientation selectivity can develop normally in PMLS without the inputs from areas 17 and 18. The development of a new specialization with cells of high spatial 'acuity', however, seems to depend on functional input from areas 17 and 18.

427.20 ADULT-LIKE PROPERTIES OF INFERIOR TEMPORAL NEURONS IN MACAQUES 4-6 MONTHS OF AGE. R.B. Rodman, C.G. Gross and J.P. Sholl*. Dept. of Psychology, University of South Dakota, Vermillion, SD, 57069.

Inferior temporal (IT) cortex is crucial for normal visual pattern perception and learning in adult macaque monkeys. However, these visual abilities, and their dependence on IT cortex, are not fully established until after the first twelve months of life. We conducted an MEG study of such lesions on macaque monkeys. We found that changes in macaque cortex persist well into the first year. These observations suggest that IT cortex undergoes significant functional maturation over the first year of life. We suggest that the possibility that developmental changes in the properties of IT neurons may underlie the development of visual pattern perception and learning. To address this possibility, we have begun studying IT responses in infant monkeys and comparing them to the properties of IT neurons in older subjects.

The activity of over 80 IT units was studied in three female Macaca fascicularis ranging in age from 115 to 195 days of age at the time of recording. Recordings were made in repeated sessions under conditions of immobilization and kynurenate anesthesia identical to those used previously for adult animals. Units were tested with wide range of light and dark edges and with a set of complex three-dimensional objects similar to those used for studies of adult IT. Each unit was also tested with movies and photographs of monkey faces. In virtually all respects, the response properties of IT neurons in this age group closely resembled those found in the adult. The majority of IT units tested were visually driven and typically responded most strongly at the center of gaze. Most receptive fields extended into both visual half-fields, although, as in the adult, responses were usually better on the contrалateral side. Selectivity for color or shape and for particular complex objects was occasionally observed. Of particular interest, cells were found that responded preferentially or only at the right of the visual field. Several of these cells responded more strongly to profile views than to front views. As in the adult, responses tended to habituate upon repeated presentation of a given stimulus, but could be subsequently elicited following the interposition of novel stimuli and/or a long intertrial interval.

In summary, the response properties of IT neurons appear adult-like in the female macaque monkey by approximately four to six months of age on virtually all dimensions previously studied in the mature animal. We now plan to record IT activity in even younger animals in order to establish the point at which properties characteristic of the adult cortex first appear.

428.1 RECURRENT EXCITATION OF MEGIAL GIANT FIBER COLLATERALS IN THE EARTHWORM: SUPPRESSION BY A SINGLE CONDITIONING STIMULUS.

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The data of Kao & Grundfest suggest that the earthworm giant fiber (GF) can influence its own activity via the action of powerful recurrent excitatory mechanisms (J. Exp. Biol., 59, 375-389, 1977; J. Physiol., 259, 621-646, 1976). We report here that following a single GF action potential, the recurrent EPSPs (i.e., the EPSPs associated with the recurrent EPSPs) could be recorded following a single GF action potential if the saline media did not contain 5 mM glucose and was not adequately oxygenated.

Thus the recurrent excitatory circuits would appear to be blocked presynaptically by recurrent EPSPs. In several preparations (N = 25) recurrent EPSPs could last for 8-12 msec and were capable of evoking a repetitive series of low amplitude dendrochemically conducted GF collateral branch spikes. These collateral branch spikes could occur at rates in excess of 800 Hz. The recurrent excitatory effects upon the GF collaterals invariably became blocked for a period of 14.8 ± 0.7 (M ± SEM) msec following a single conducted GF action potential. There was no evidence of an inhibitory post synaptic potential in the GF during the time of block, and the recurrent EPSPs always occurred at a rate of 1 action potential per 2-3 Hz. In 10/28 preparations (N = 3), the recurrent EPSPs appeared to block the behavioral response to hypoxic drive, as shown above, and further support for these observations was obtained from the observation that freshly dissected ganglia shortly after being switched to hypoxic perfusion.

We tested the hypothesis that a single conditioning stimulus could elicit the response seen above. During hyperbaric perfusion (>580 torr, 1 atmos) the response is the dominant mode. During long pauses which may last >30 min. temporary bursting is often restored by brief trains of depolarizing current pulses. However, these visual abilities, and their dependence on IT cortex, are not fully established until after the first twelve months of life. Moreover, post-natal metabolic and anatomical functional maturation within the first year of life, and raise the possibility that developmental changes in the properties of IT neurons may underlie the development of visual pattern perception and learning. To address this possibility, we have begun studying IT responses in infant monkeys and comparing them to the properties of IT neurons in older subjects. The activity of over 80 IT units was studied in three female Macaca fascicularis ranging in age from 115 to 195 days of age at the time of recording. Recordings were made in repeated sessions under conditions of immobilization and kynurenate anesthesia identical to those used previously for adult animals. Units were tested with wide range of light and dark edges and with a set of complex three-dimensional objects similar to those used for studies of adult IT. Each unit was also tested with movies and photographs of monkey faces. In virtually all respects, the response properties of IT neurons in this age group closely resembled those found in the adult. The majority of IT units tested were visually driven and typically responded most strongly at the center of gaze. The receptive fields extended into both visual half-fields, although, as in the adult, responses were usually better on the contralateral side. Selectivity for color or shape and for particular complex objects was occasionally observed. Of particular interest, cells were found that responded preferentially or only at the right of the visual field. These cells responded more strongly to profile views than to front views. As in the adult, responses tended to habituate upon repeated presentation of a given stimulus, but could be subsequently elicited following the interposition of novel stimuli and/or a long intertrial interval.

In summary, the response properties of IT neurons appear adult-like in the female macaque monkey by approximately four to six months of age on virtually all dimensions previously studied in the mature animal. We now plan to record IT activity in even younger animals in order to establish the point at which properties characteristic of the adult cortex first appear.

428.2 RESPONSES OF THE ISOLATED CRAB VENTILATORY CPG TO VARIATIONS IN P02. J.L. Wilkens, R.E. Young* and R.A. DiCaprio, Biology, Univ. of Calgary, Canada; Physiology, Univ. West Indies, Jamaica; & Zoological and Biomed Sci, Ohio Univ, Athens, Ohio.

Ventilation of intact crabs, Carcinus maenas, in air-equil (SW) or hypoxic saline (SC) beating alternating with short and long pauses. In hypoxic SW (<400 torr) pausing predominates, while in hypoxic (500 torr) stimulates hyperventilation without pauses (compensatory responses which may persist for days), and ventilation is slow and regular in deep hypoxic (SC) breathing. Ventilatory burst rates (BRC) recorded from the SC motoneurons of the in vitro perfused thoracic ganglion are proportional to P02, particularly after 60 min exposures. This trend, but with greater variability, also holds for 15 and 30 min exposures.

During hyperbaric perfusion (>580 torr, 1 atmos) pausing often alternates with rapid bursting, with pausing sometimes being the dominant mode. During long pauses which may last >30 min. temporary bursting is often restored by brief trains of depolarizing current pulses. These responses are similar to those of intact crabs.

Upon switching to air-equil or hypoxic saline, BRC's steadily decline from 60-150/sec and may not stabilize by 60 min. At 135 torr, pauses occur infrequently and are generally of shorter duration than at 600 torr. In hypoxia, pausing is not observed and bursting is slow and regular. Even though rates are depressed, the deafferented ventilatory CPG's appear to respond to hypoxic drive, as shown above, and further support for these observations was obtained from the observation that freshly dissected ganglia shortly after being switched to hypoxic perfusion.

"Compensatory" accelerated BRC's lasting ~10 min are occasionally seen following perfusion at lower P02. Response latencies following switches in perfusion are 20 sec or less. BRC's are often higher (repayment of 0 debt?) after return to hyperbaric saline following a period at lower P02.
428.3 AMINERGIC MODULATION OF GRADED CHEMICAL AND ELECTRICAL SYNAPTIC EFFICACIES WITHIN THE PYLORIC MOTOR CIRCUIT OF THE LOBSTER: GRADED CHEMICAL SYNAPTIC EFFICACIES UNDERLY THE COMPOSITE DISCHARGE OF A SINGLE IDENTIFIED NEURON EXERTS SUPPRESSIVE MODULATORY CONTROL OF A LOBSTER CENTRAL PATTERN GENERATOR

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Production of rhythmic motor activity by central pattern generators (CPG) is controlled by neuromodulators, as has been shown clearly in the stomatogastric nervous system of Crustacea. In this system, however, the roles of these two factors are not as clear. The neuromodulators described so far act in a permissive way, i.e. they initiate or enhance the bursting properties of target networks. In contrast, the pyloric CPG in the stomatogastric ganglion (STG) of Homarus also receives an inhibitory control which is capable of generating full bilateral flexion or extension when depolarized. The roles of the two factors are unusual, however, in that the depolarizations due to sequential membrane properties and due to synaptic input occur sequentially rather than at the same time, as is the case for most neuromodulators studied to date. This result in a composite discharge in LG, comprised of two distinct phases of firing and a period of silence. The first firing phase is dependent on a 2 Hz endogenous component of LG firing, and the second is largely due to excitatory synaptic input from a rhythmic extrinsic input, the pyloric suppressor (PS) neuron, that we have identified. The PS cell body is located near the esophageal ganglion, and projects to the STG. When PS is induced to fire a burst of action potentials, the ongoing pyloric activity is considerably disrupted. A 3 sec. discharge of PS, for example, is able to stop pyloric rhythmicity for more than 60 sec. Moreover, the different pyloric neurons display different sensitivities to PS effects; the LP neuron being the most sensitive, the PD neuron less so, and the neurons controlling the pyloric valve, the PD and LP, being almost insensitive. As a result, PS activity can not only stop pyloric rhythmicity for more than 60 sec. At the same time, it also causes long-lasting modifications in the structure of the pyloric sequence.

PS tonic discharges induce graded alterations in the pyloric pattern that depend on the PS discharge frequency. Up to 5 Hz, PS discharge gradually changes the phase relationships and intensities of the pyloric neurons’ bursts. Due to the different sensitivities of the pyloric neurons to PS effects, some of them can be selectively switched off. For instance, a 2 Hz variation in discharge of PS can induce a change from a triphasic to a biphasic pattern by silent neurons (Hasler and Wersing, 1985), and a further increase to 10 Hz may elicit PS effects on the pyloric pattern are partly due to the long lasting suppression of the ability of the pacemaker and LP neurons to produce bursting pacemaker potentials.

In conclusion, by controlling the endogenous properties of some pyloric neurons, the modulatory PS neuron can either provoke a long-lasting suppression of the pyloric rhythm or modify the pyloric motor pattern in a graded fashion. In particular, by controlling the number of neurons that participate in the pyloric rhythm, PS can use the pyloric neuronal network to assume different functional configurations. Small variations in PS firing produce shifts from one functional configuration to another.
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The marine gastropod mollusc Pleurobranchaea exhibits many different oral behaviors using similar sets of muscles and nerves. One class of neurons, the buccal cerebral neurons (BCNs), lying in the buccal ganglion and projecting to the cerebropleural ganglion, has a pivotal position in the generation of all behaviors requiring coordination between the jaw/radula and the mouth and lips (Miltosev, G. J., and Cohen, C. S., J. Neurobiol., 17: 517, 1986). The activity of the BCNs and the behaviors in which the BCNs take part in generating are variable (Miltosev, G. J., and Cohan, C. S., J. Neurobiol., 17: 499, 1986). Dimensional analysis of the spike series in the BCNs, and in the motoneurons that they drive, indicates that the dynamics of the activity of the BCNs and the behaviors in which the BCNs take part in generating are variable (Miltosev, G. J., and Cohan, C. S., J. Neurobiol., 17: 499, 1986).

428.8 MYTHS AND REALITIES OF FREQUENCY GRADIENTS AND CONSTANT PHASE LAGS DURING LAMPREY FICTIVE SWIMMING. A. H. Cohen and M. W. Baker.*
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Several investigators have characterized fictive swimming in the isolated spinal cord of the lamprey (e.g., refs 1,2). It has been said that one characteristic of the motor pattern is that the bursts of two ventral roots (VR) are separated by a constant phase delay of 18 of the cycle per segment as is seen in intact animals. The evidence for this in terms of extracellular recordings of widely separated VRs. We now report that when the phase delays are computed between VRs for more than 5 segments apart, that the delays along the cord are not constant. We can also report that when the gradient of preferred segmental oscillator frequencies is examined in detail, in the majority of spinal cords it is not as (some of us had suggested) monotonic. The phase lags were examined in isolated spinal cord-cutout preparations of lamprey somites 1-10. Fifty segment cord pieces (of the 100 total in the intact animal) were activated by bath application of D-Glucose (125-500µM). A method was devised whereby delays among neighboring segments could be measured; this was used along the entire length of the cord piece. The results revealed that that in the middle 20 segments as well as at the rostral and caudal ends of the cord the phase delays were positive, but not uniformly 18 of the cycle per segment. Approximately 10 segments from either end of the cord and the phase delays became negative (1-3 of the cycle per segment). Among the segments just rostral and caudal to the negativity, the delays rose above 18. This pattern was seen in all the cord tested to date regardless of the pattern of segmental frequencies they exhibited.

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428.9 COMPARISON OF FORWARD AND BACKWARD WALKING IN CHICKS. A. Belfot, P. Fuscht, R.M. Johnston* and H.E. Rettie.* EPO Biology Dept., University of Colorado, Boulder, CO 80309.

We have examined forward and backward walking in chicks to determine whether hip muscle activity patterns show a phase shift of 0.5. EMG electrodes were implanted in 6 leg muscles of 0-6 day-old chicks: gasternoenemius lateralis (GL), an ankle extensor; iliofibularis (IF, a knee extensor), caudofibularis (CF, a hip extensor and knee flexor) and sartorius (SA, a hip flexor and weak knee extensor). Recordings were made during forward and backward walking on a treadmill as well as during free walking.

Numerous differences are seen in cycle period, burst duration and phase of activation of the muscles. However, although phase shifts in hip muscle activity are seen, they are far less than 0.5. That is, the hip extensors remains coactive with knee and ankle extensors bursts in forward walking as well as for forward walking, which is currently underway, will allow us to relate the EMG patterns to the actual movements performed during forward and backward walking. Supported by NIH grant NS 20310.
428.11 COMPARISON OF STEP CYCLE TIMING IN THE ISOLATED MOUSE CORD-HINDLIMB PREPARATION WITH THAT IN INTACT, PARTIALLY MUSCULARIZED. M.B. Droge, S.L. Stewart* and P. Herrild*. Dept. of Biology, Texas Woman's University, Denton, TX 76204.

From previous ventral root recordings, we know that combined spinal cord-hindlimb explants from neonatal mice (5-6 days old) are capable of generating certain motor rhythms (Droge et al., Brain Res., submitted) and that these patterns are similar to those recorded from dissociated cell cultures of fetal mouse spinal tissue (Droge et al., J. Neurosci. 6(6): 1583, 1986). The objective of the present study is to relate the timing of a normal step cycle in freely moving neonatal and adult mice to the rhythmic motor activity observed in the explant preparation.

Electromyographic (EMG) recordings were made primarily from groups of tibialis anterior muscles of neonatal and adult Balb/C mice. The neonatal nerves were from the same age group as those used for the explant studies (birth to 5 days old). In some cases, data for the intact and explant preparations were obtained from the same animal. Electrode placements remained the same. The normal step cycle in intact neonates ranged from 300-1100 ms, which was comparable to motor rhythms recorded from the explanted system. Spontaneous stepping, however, occurred far less often in the isolated spinal cord-hindlimb preparation and the cycle periods tended to be at the slower end of the range found for intact neonates. Compared with neonates of either group, freely moving adult mice tended to have faster step cycles with a range of 150-650 ms.

These data demonstrate that an isolated spinal cord-hindlimb of an adult mouse can generate normal step cycles for that age group. Although these trends tend to be slower than those of freely moving adult mice, there is enough evidence to suggest an important link between the intact adult and the explant preparation. In addition to characterizing further the timing of the step cycles, we are also pursuing a systematic recording of the isolated mouse spinal cord to determine the minimal lumbosacral excitations capable of pattern generation.

428.12 Voltage clamp frequency domain analysis of NMDA activated spinal neurons. L. E. Moore, R. R. Hill & S. Grillner, Nobel Institute for Neurophysiology, Karolinska Institute, Stockholm, Sweden and Department of Physiology & Biophysics, University of Texas Medical Branch, Galveston, Texas 77550, U.S.A.

Most studies of the quantitative effects of excitatory amino acids on receptor subtypes has generally been restricted to isolated or cultured neurons. The recent advances in the knowledge of the mechanisms involved in the mechanisms of amino acid receptors and the fact that they have been used to stimulate locomotor-like behavior. The receptor subtype responding to N-methyl-D-aspartate (NMDA) of the lameness spinal cord fulfills this condition by activating line running movements. The receptor afferents are likely to be TTX (Brodin, Grillner, & Rovainen, 1986; Dale & Grillner, 1986). NMDA induced oscillations are oscillations in the presence of TTX (Brodin & Grillner, 1986) are essentially abolished by the potential control of the voltage clamp. Therefore, a number of the receptor sites responsible for the oscillations are electrochemically close to the soma. In the presence of NMDA and TTX set steady state inward currents can be observed at moderate depolarizations. Under these conditions, current voltage plots reveal a negative slope conductance in the potential range of the inherent oscillations. A kinetic analysis of the voltage dependent conductances is possible if the electrotonic structure of the cell is taken into account. Such an analysis was done with a frequency domain technique (Moore & Christensen, 1985) using a white noise stimulus to linearly perturb the membrane potential over a wide range of frequencies. The analysis revealed that the negative conductance leads to a response which is nearly 180 degrees out of phase with the stimulus. This is an unstable condition which leads to the dephosphorylation of the induced oscillations. The phase-reversing phase is the result of the activation of a positive conductance which is a resonance suggesting that an oscillating neuron can inherently exhibit certain frequencies. Measured impedance functions show a nonlinearity in the voltage and resonance behavior and yielded phase behavior characteristic of a negative conductance. Synaptic integrative responses were evaluated by measuring impulse responses from the measured transfer functions.

428.15 THREE-DIMENSIONAL GRAPHICS FACILITATES USE OF INTERACTIVE NEURAL NETWORK COMPUTER SIMULATION. C.D. Myers*, W.K. Smith and D.J. Woodcock, Dept. of Cell Biology and Anatomy, Univ. of Texas Health Sci. Ctr. at Dallas, Texas 75235.

Advances in the theory of neural networks have implications for understanding the functioning of the nervous system. Our experiences in neural network simulation suggest the need for a graphics processing environment for modeling and a graphics display to study the properties of these models. Ongoing studies in this laboratory have explored the application of three-dimensional and graphic techniques in a visualization analysis of neural networks through the development of the program CARMAC (Computer Aided Reconstruction Package). This package provides graphics tools for the display of three-dimensional phenomena, as occur in neural networks. We have designed a neural network simulation package called NET which supports models based on the principles found in contemporary research on parallel distributed processing. The NET design provides for the generation and editing of a variety of models. Generation of a particular model requires the user to define a set of connected conceptual units, each unit of which is made up of inputs, connection weights, an activation value, and an output. If units are thought to represent neurons, inputs might be defined as the incoming firing rate to the neuron, weights defined as the effectiveness of a synapse on the neuron, activation values defined as the sum of the effects of the weighted inputs on the neuron, and the output defined as the firing rate of the neuron. The equations for the simulation of the model rule how these parameters interact, and the editing facility allows the user to change selected parameters at any time.

Three-dimensional graphic representations of the dynamic characteristics of units in a simulation can be used to visualize the parameters associated with units: action potentials, membrane time and space constants, ionic currents, flow, etc.) in the simulation package. We will also assess the use of multiple processor hardware as a host for the simulation package. Support from the Biological Humanities Foundation.

428.16 DARWIN MACHINES: SELECTION AMONG STOCHASTIC SEQUENCES IN PARALLEL. William H. Calvin, University of Washington, Biologics Program, NZ-15, Seattle, WA 98195.

Darwin Machines are my name for a class of computational automata (both biological and artificial) which might be used to promote the highest grade being selected to be let loose on the main track (or if paper is constructed to be selected for a stage). These candidate sequences might be modular command concepts for a ballistics movement such as throwing or hammering (which require serial command because feedback is too slow to be effective). The selection of the most probable sentence/particle schemes for constructing a scenario that connects past and future in some sequence (if well-ordered), we may call this logic: if grading against a memory is slipshod, the resulting sequences may be no more orderly than one's dreams.

The random or stochastic selection of somatic units (many tracks) and many repeated cycles which shape up survivors before overt action is taken. Just as many body structures logic of the beginning but in this case 'survive' to the highest grade. It is initially the same, too logical to have arisen by chance, so our best thoughts may be few (or remaining signs of their stochastic sequencer origins (except, interestingly, for our notion of 'free will' -- the perception that we actively make choices which is a natural outcome of the differences). The premonitor cortex has the motor sequencing which suggests a 'final common path' for this sort of stochastic sequencing. The candidate sequences are constructed for temporal and/or temporal selections, even with basal ganglia and cerebellum. Just as in biological evolution, evolutionary phenomena in neuromuscular units (action potentials, membrane time and space constants, ionic currents, flow, etc.) in the simulation package. We will also assess the use of multiple processor hardware as a host for the simulation package. Support from the Biological Humanities Foundation.


The protocol for conditioning of opiate withdrawal was almost identical to that previously used for place conditioning (Mucha et al., 1986), except that animals could not be conditioned to the environment but only to two objects (a 2.5 cm sphere and a similarly-sized cube). Rats were first trained to press for saline (1 mg/kg) or cocaine (10 mg/kg) without any stimulation. Preliminary studies indicated that cocaine alone, (similar to London et al., Neurosci. Lett., 61(3), 1986), decreases metabolism in the lateral habenula, and it may slightly increase globus pallidus and substantia nigra metabolism. Self-stimulation/saline water was administered to Esk et al., (Proc. Natl. Acad. Sci., 81, 3635, 1984) appears to increase metabolism in the ventral tegmental area, substantia nigra, dorsal raphe, and substantia nigra metabolism is not different from unstimulated controls. In rats given cocaine and stimulation, metabolism may be decreased in the lateral habenula (and the dorsal raphe as compared to saline/stimulation rats). These metabolic rates are lower than those in cocaine, stimulated rats. Metabolism in the globus pallidus may be increased in cocaine/stimulation rats, beyond the increases seen in saline/stimulation or cocaine only rats. Additional data work confirms or refutes these preliminary results. Supported by grants MW50507 and #G273216.

A recent mapping study has shown the existence of a high concentration of reward-relevant neurons in the medial mesencephalon (Rompré and Wise, Brain Res. 246:129, 1982). The results of this mapping study raise the possibility that reward-relevant neurons directly link the ventral tegmental area and the caudal mesencephalon. A one-way testing of this hypothesis is to use the behavioral adaptation of the collision technique developed by Shizgal and colleagues (J. Comp. Physiol. Psychol. 96: 277-279, 1982) to determine whether the axons of reward-relevant neurons directly link two distinct brain sites that support self-stimulation. They used the collision method to demonstrate an axonal link between the lateral hypothalamus and the ventral tegmental area. The aim of the present study was to extend this finding to other sites. Direct axonal link between reward-relevant neurons in the ventral tegmental area and the caudal mesencephalon. Male hooded rats were implanted with a fixed monopolar electrode in the ventral tegmental area, and a moveable electrode in the caudal mesencephalon. The collision test was performed once self-stimulation behavior was stable on both electrodes. The collision test consisted of the delivery of trains of pairs of stimulating pulses, a conditioning or C pulse and a test or T pulse. The C pulse was delivered to one electrode, and the T pulse to the other electrode. The interval between C and T pulses was varied between 0.2 and 10 msec. Collision was inferred from a significant increase in self-stimulation threshold at short C-T intervals. If collision was not found at the first tested site on the moveable electrode, the electrode was lowered by 0.16 mm and a new collision test was performed. Evidence of collision was observed in six animals the self-stimulation threshold increased at C-T intervals shorter than 1.2 msec. Histological analysis performed on five brains revealed that all but one rostral electrode was located in the ventral tegmental area and the caudal electrodes were located in the medial mesencephalon, in or near the central gray. The remaining rostral electrode was found in the posterior hypothalamus. These results provide direct axonal link between reward-relevant neurons in the caudal mesencephalon and the ventral tegmental area-posterior hypothalamic.

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Using the behavioral adaptation of the collision technique developed by Shizgal and colleagues (J. Comp. Physiol. Psychol. 96: 277-279, 1982), we demonstrated an axonal link between the reward-relevant neurons in the ventral tegmental area and the caudal mesencephalon (Boye and Rompré, Soc. Neurosci. Abstr. 13, 1987). The behavioral collision test consists of delivering trains of pairs of stimulating pulses, a conditioning or "C" pulse and a test or "T" pulse. The C pulse is delivered to one electrode, and the T pulse to the other electrode in alternating fashion. The interval between C and T pulses is varied and collision is inferred from a significant increase in the conduction time of the T pulse. Estimates of the conduction velocity can be obtained by dividing the difference between the collision interval and the refractory period. In this study, refractory periods were estimated from five animals that showed a collision effect. The results show that the conduction velocity is similar in both the analogous paradigm but with both pulses applied through the same electrode. Recovery from refractoriness was first evident at C-T intervals greater than 0.5 and 0.6 ms. Collision velocity estimates obtained for each subject range from 4 to 10 m/s, values consistent with the estimates reported for the ascending noradrenergic fibers. This finding that at least part of the substrate for brain stimulation reward that links the ventral tegmental area and the caudal mesencephalon is composed of non-adrenergic, myelinated axons.

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In the present study spontaneous motor activity in rats and mice was measured with a Digiscan Animal Activity Monitor (Omnitech Electronics, Columbus, OH). Male hooded rats (n = 24) were tested for 4 hours/day, 3 days per week. The Digiscan System differentiated 9 aspects of the spontaneous behavior: horizontal activity (HA) - total number of photobeam interruptions, total distance travelled (TD), movement time (MT), average speed (AS), number of horizontal movements (NM), average distance of each movement (AD), vertical activity (VA), number of beam interruptions, vertical movement time (VT), and number of vertical movements (NVM). Most of the variables exhibited significant (p < .05) decrements in magnitude (habituation) over weeks of testing (except NM, AD, and AD). Reliabilities of the measures aggregated on a weekly basis were high (r > .45) except for HA and VA. Intercorrelations of the variables tended to be large (r > .6) and positive except for NM, which exhibited a peculiar pattern of correlation with the other variables. Male CF-1 adult mice (n = 30) were also tested for 1 hour, 2 days per week for 3 consecutive weeks. The same variables were recorded in the Digiscan System, except that VA, VT, and NVM were not used because the mice were too small to activate the vertical sensors. The mice exhibited a different pattern of habitation in activity relative to the rats. For HA, AD, and VA habituation (p < .05) tended to be large in the first week with smaller values of the behavioral measures showing good stability in weeks 2 and 3. The reliability of the variables (weekly aggregates) were greater for mice (relative to the rats) for all variables. Intercorrelations of the variables also tended to be large and positive except for NM, which again exhibited a different pattern of correlation in relation to the other variables. This pattern was similar to the one found in the rat data.

The present results indicate that habituation to the apparatus is necessary to obtain stable baseline data, that rats habituate more slowly than mice, that most of the variables show good re-test reliabilities, and that intercorrelations between the variables are high. These results suggest that at least part of the substrate for brain stimulation reward that links the ventral tegmental area and the caudal mesencephalon is composed of non-adrenergic, myelinated axons. (Supported by a Natural Sciences and Engineering Research Council grant, A1239, to KPO.)

The effects of cholinergic manipulations on the rewarding consequences of self-stimulation (self-S) have been described in many studies, usually in negative terms. However, more recent reports suggest that the use of behavioral methods yields more sensitive measures for the action of acetylcholine (ACh) on self-S and other behaviors.

In the present study, we have investigated the effects of atropine (0.5 to 10 mg/kg, IP) on the reward-related processes of operant behavior in rats. Atropine (0.1-5 mg/kg, IP) was found to significantly decrease the number of pulses per train of constant duration. Measurements were repeated under vehicle, atropine sulfate (0.5 to 10 mg/kg, IP), or scopolamine (0.5 to 4.0 mg/kg, IP). No significant shifts in the function were seen to occur at any dose of either drug. The results support the hypothesis that cholinergic blockade results in the inactivation of a proportion of the directly stimulated elements. This hypothesis is consistent with the findings of earlier studies involving receptor antagonists with the present findings on reinforcer functions.

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Many disorders of calcium metabolism are associated with mood changes that are similar to those of affective illness. Furthermore, in such disorders the restoration of calcium levels to normal is accompanied by a change in mood. Cogan et al. (Am. J. Med., 65, 1976, 963), for example, reported a significant improvement in mood in patients with secondary hyperparathyroidism after parathyroidectomy. Also, patients with primary depression often exhibit altered levels of calcium in the cerebrospinal fluid and plasma. The recovery from depression, on the other hand, is associated with the return of calcium levels to normal. Since calcium regulates the synthesis and release of neurotransmitters postulated to be involved in the etiology of mood disorders, as well as altering the responsiveness of postsynaptic receptors, altered calcium levels may be involved in mood disorders. In the present study, depressed human subjects, as defined by a score of 15 or more on the Hamilton Depression Scale, received either calcium gluconate (1000 mg) containing vitamin D (60 I.U.), or placebo twice daily for four consecutive weeks. Before and after scores on the Beck Depression Inventory (BDI) indicated that subjects who received calcium plus vitamin D showed a significant elevation in mood as compared to the placebo group. Analysis of variance (ANOVA) on the data from the 67 participants revealed no significant difference between the calcium and placebo groups on the BDI (calcium group = 16.9 ± 2.1; placebo = 15.8 ± 1.0) upon the first administration, i.e., prior to experimental manipulation. When ANOVA was performed on the second administration of the BDI after the completion of the first injection of calcium plus vitamin D, it was statistically significant with a lower (i.e., enhanced mood) BDI score in the calcium group (3.9 ± 0.9 vs 14.6 ± 2.4 in the placebo group). The subjects who received calcium plus vitamin D showed significantly higher plasma calcium levels (calcium group = 11.4 ± 0.1 mg/dl; placebo group = 9.4 ± 0.1 mg/dl, P < 0.01, two-tailed t-test). Thus a strong tendency for calcium plus vitamin D supplements to elevate mood was observed in the present study. The effects of previously found that calcium alone does not elevate mood in subjects with normal BDI scores. Therefore, calcium only appears to be effective in patients who have a more severe form of depression, leaving the non-depressed individuals unaffected. This suggests a role of calcium in the therapy of depression and possibly in its etiology as well.

429.9 HEROIN SELF-ADMINISTRATION IN THE RAT SUPPRESSED BY SCH 23390. A. Nakajima and B. K. Page Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Quebec, Canada.

Operant responding for various reinforcer agents can be suppressed by an i.p. injection of SCH 23390, a selective dopamine D1 receptor antagonist. In the present study, we report a suppression of bar-pressing response reinforced with heroin injection using 16 infrared lightbeams in a Digiscan Monitor (Omnitech Electronics, Ltd.). The floor of the square recording cage was divided into 4 quadrants and animals were injected with either saline or SCH 23390 (0.04-0.08 mg/kg, IP) at a volume of 0.25 ml/kg. The resultant current-frequency trade off function provides an index of temporal and spatial summation within the substrate (Gallistel et al., Psych. Rev., 1981, 88, 218). The hypothesis that dopamine D1 blockade results in the inactivation of a proportion of directly stimulated elements was tested by obtaining current-frequency functions under vehicle or atropine (0.5 to 10 mg/kg, IP). No effects of the drug could be detected on the scale or shape of the functions. Considered together, the two experiments do support the assertion that in the lower dose range of SCH 23390, the drug interferes with the action of dopamine on the reward-related processes of operant behavior in rats.

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Injections of carbachol (CCl) into the anterior hypothalamic/preptic area decrease locomotor activity and rearing behavior in rats. We have recently observed that the pattern of locomotor behavior is also altered by these injections. Thus, in the present study, we have examined the effect of injections of CCl on activity cage during undisturbed behavior and after intracerebral injections of CCl.

Eighteen male rats were bilaterally implanted with stainless steel cannulae for injections of CCl into the anterior hypothalamic/preptic area in chronic experiments. Cannulae were inserted into the brain 7-8 mm anterior to the interaural plane according to the Paxinos and Watson stereotaxic atlas. Activity of rats was recorded using 16 infrared lightemitters in a Digiscan Monitor (Omnitech Electronics, Ltd.). The floor of the square recording cage was divided into 9 zones. Locomotor activity and time spent in each of these zones were measured for 5 min before and 10 min (25 sec) after injection of CCl or saline as a control. All the cannula placements were verified histologically post-mortem. A total of 36 injection sites in the hypothalamic/preptic area were explored and injected with CCl (1 µg in 0.2 µl) and isotonic saline (0.2 µl) in random order.

Freely behaving rats spent 85.7% (257.0 ± 32.0 sec) of the time in all corners (corner time), 14.2% (42.6 ± 32.1 sec) in the central zone, and 0.2% (0.4 ± 1.5 sec) in the central zone. The injection of CCl into a fixed corner decreased significantly the corner time in the first 5 min post-injection to 61.7% (185.2 ± 63.4 s; t-test, n=36, p < 0.001) and the corner time in the first 5 min post-injection to 61.7% (185.2 ± 63.4 s; t-test, n=36, p<0.001) and the corner time in the first 5 min before injection. Injection of isotonic saline had no change in corner time. However, the injection of CCl into the same brain sites decreased significantly the corner time in the first 5 min post-injection to 61.7% (185.2 ± 63.4 s; t-test, n=36, p<0.001) and the corner time in the first 5 min before injection. The effect of CCl was dependent on the location of injection site and was not obtained from more rostral areas than 8.5 mm anterior to the interaural plane. Changes in spatial pattern of rat behavior induced by injection of CCl were usually concomitant with a decrease in locomotor activity. Both these behavioral manipulations were reversed with pretreatment with 1.5 µg of atropine.

The results are interpreted in terms of cholinergically-evoked changes in locomotor and emotional components of adaptive behavior.

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429.11 SENSITIZATION OF SELF-STIMULATION AND SPONTANEOUS MOTOR ACTIVITY REDUCTIONS AFTER CHRONIC LOW-DOSE HALOPERIDOL TREATMENT. M. Lynch, L. Lynch, S. Keery, Res Dev Dept and Dept of Psychiatry, V A Med Ctr and SUNY Health Sci Ctr, Syracuse, NY 13210

Previous studies have suggested development of tolerance to the neuroleptics’ rate-suppressing effects on self-stimulation (SS) behavior (Fichet et al, Neurosci., 2:846,1976). As these studies employed high doses and examined only response rate (versus threshold) measures, it would seem that these data reflect an adaptation to neuroleptic-induced motor impairment similar to that which has been observed in other systems. We have previously demonstrated that low dose (0.1 mg/kg) haloperidol (H) treatment produces changes in both threshold and asymptotic performance dimensions of rate-intensity functions (Lynch & Lynch, Psychopharmacol., 12:937,1979). Therefore, VTA-implanted rats (n=5) were given 0.1 mg/kg H once per day for 10 days in order to track effects of chronic neuroleptic treatment in a dose range that produces stable behavioral changes indicative of altered reward substrate (H-pre group). Behavioral testing was conducted every 1 or 2 h after Control rats (n=6) received H after each behavioral test (H-post). Surprisingly, H-pre rats showed an almost complete suppression of SS upon the first H administration, which became even more pronounced by the second test. The acute result was unexpected from our previous observations and it is not clear why inconsistent findings have been reported across groups of animals and test procedures for similar doses of this drug (see e.g., Lynch & Wise, PNB, 23:777,1986). Tolerance was not observed over repeated administration and testing. In fact, concomitant observations of spontaneous motor activity in an open field revealed sensitization to H’s rate-reducing effects that were output in the H-post group treated at baseline for both behavioral measures. After 10 days of 0.1 mg/kg H, both groups were tested with a series of low doses of H (.025 to 0.1 mg/kg) administered 1 h prior to testing. Although the groups had similar pharmacological histories, repeated reductions in the previous H-post group were very small (an approx. 10% reduction in peak SS rates), while H-pre rats exhibited increases in peak rates and much lower rates of spontaneous motor activity as well). Decreased asymptotic performance was observed in the absence of reliable threshold shifts that would indicate performance changes. In conclusion, low dose H treatment paired with the SS test chamber contributed to greater response reduction upon subsequent testing with 0.025 to 0.1 mg/kg H and produced sensitization to reduce spontaneous motor activity that persisted after the chronic treatment with 0.1 H. This environment-expanded neuroleptic-induced deficit may be of relevance for interpreting delayed-onset therapeutic and motor effects with clinical usage. Supported by N.I.R.A. Research Service Award 1 F32 DA00291-01A2 NMR-1 to N.R. Lynch.

429.12 CHRONICALLY ISOLATED RATS INTRAVENOUSLY SELF-ADMINISTER MORE COCAINE BUT NOT AMPHETAMINE WHEN COMPARED TO CHRONICALLY ISOLATED RATS. E. Schiller and E. Amst, CSSM, Concordia Univ., Montreal, Quebec, H3G 2M8.

We have been studying the role that environmental factors play in the predisposition to drug abuse. Sensitivity to a number of abused drugs, as measured by a number of behavioral paradigms, is markedly influenced by an early housing manipulation consisting of isolation or aggregation in weaning rats. These conditions, however, do not effect the sensitivity to amphetamine, as measured by place conditioning to the previously rewarded stimulus (NSERC). We have extended our previous finding that SNpc, a region of the ventral midbrain, is extremely sensitive to amphetamine (chronic i.v. injection) by examining the propensity of isolated or aggregated rats to intravenously self-administer cocaine or amphetamine.

Male Long Evans rats were obtained at weaning (21 days) and were housed either singly or in groups of 4 for 6 weeks. They were then prepared with chronic, indwelling jugular catheters. Following one week, the rats were introduced to an operant chamber where depression of a lever resulted in a 0.1 ml infusion of cocaine (10 or 0.1 mg/kg) or saline (0.1 ml). A repeated measure design was used whereby “coca ine” rats were tested with 1.0, 0.5 and 0.1 mg/kg/infusion and “amphetamine” rats were tested with 0.25, 0.125, 0.0625, 0.0313, or 0.0125 mg/kg/infusion. Doses were maintained for 2-4 days and were run in descending order. These sessions were 3 hours long. Except for this daily 3 hr period, housing conditions were maintained.

The isolated rats self-administered cocaine in a dose-dependent fashion, with maximal rates obtained at the 0.5 mg/kg dose. The groupd rats failed to reliably self-administer this drug regardless of dose. In contrast, rats from both housing conditions self-administered amphetamine, dose-dependently, with maximal rates at the 0.06 mg/kg dose.

These data suggest the suggestion that the reinforcing properties of only some drugs of abuse are influenced by environmental factors. Further, the difference in the effect on cocaine and amphetamine self-administration may reflect specific neurochemical changes associated with the manipulation.

429.13 IN VIVO ELECTROCHEMICAL EVIDENCE FOR RELEASE OF Dopamine Evoked BY REWARDING ELECTRICAL STIMULATION. A. Gratton, G. Gerhardt and R.J. Hoffer, Dept. of Pharmacology, U. of Colorado Hlth. Sci. Ctr., Denver, CO 80262

There is now considerable psychopharmacological evidence implicating the A10 dopamine (DA) system in brain stimulation reward (BSR). However, more recent psychopharmacological data indicate that DA fibers are not directly activated with stimulation parameters commonly used to elicit BSR. Rather, it seems that they may be either insufficient to the directly activated substrate of BSR or may in some way modulate the activity of the BSR substrate. While many previous studies have showed forebrain burst (NBF) electrically-evoked release of DA, the strength of the stimulation used in these studies was often several fold greater than what is required to elicit a rewarding effect. The present study now provides preliminary data suggesting that rewarding levels of MFB stimulation do elicit small but detectable increases of DA release as measured by in vivo high speed chronocoulometry.

Male Long-Evans rats previously trained to lever-press for rewarding stimulation applied to either the NBF or the ventral tegmental area (VTA) were anesthetized with urethane and placed in a stereotaxic apparatus. A Nafion-coated graphite/equivalent cell (GEC) electrode was then lowered into the various DA terminal fields. Chronoamperometric measurements were obtained by applying square-wave pulses (0.5 to 3 Hz, 80 mV peak-to-peak) from the GEC at a rate of 5 or 10 Hz. The resulting current flow generated by the oxidation and subsequent reduction of electroactive species at the OEC tip was digitized and integrated over the last 60% of the positive and negative going pulses. Changes in DA concentration were monitored in a warmed (37° C) glass chamber (0.1 ml) in 0.1 nmoles cathodal pulses were applied to the NBF or VTA at frequencies in the 20-100 Hz range and at current intensities of 500-100 uamp. The magnitude of the DA responses was positively related to the magnitude (charge) of the stimulation. The duration of DA release generally occurred in 5-6 sec. Such rapid changes were detected in the nucleus accumbens, caudate nucleus, prefrontal and cingulate cortex. While these data do not prove that the electrically-evoked release of DA is reward-related, we conclude that electrode-evoked DA release by the experimenters' application to an electroactive substrate and VTA sites is capable of eliciting measurable increases of DA in the extracellular space.

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429.14 DISASSOCIATION OF MORPHINE’S DISCRIMINATIVE STIMULUS PROPERTIES FROM ITS MOTIVATIONAL PROPERTIES. G.M.Martins, A. Barch and D.J. van der Ende, Dept. of Psychology, Memorial University of Newfoundland and Dept. of Anatomy, University of Toronto, Toronto, ON, Canada M5S 1A8

Opiates have both positive reinforcing and aversive motivational properties. In addition, morphine has cueing properties, in that it can serve as a discriminative stimulus. We investigated whether rats use the positive reinforcing or aversive motivational properties of morphine as the perceived property that controls conditional responses. We show that morphine acquires discriminative properties in as little as 5 pairings with a novel solution and an appropriate unconditional stimulus when we use a recently described conditioning procedure (Wall & Riley, Psychon. Soc. Meeting, 27,18,1986). On alternating days, rats were injected with either 5 mg/kg of morphine or its vehicle 15 min prior to access to a 1.7% vinegar solution. On morphine days an i.p. injection of 130 mg/kg of lithium chloride immediately followed the end of the 15 min exposure to the vinegar. After the course of 5 morphine trials the rats learned to avoid vinegar on morphine days and to consume vinegar on vehicle days. Control experiments showed that the rats had learned a robust discrimination that could not be attributed to non-specific effects such as the injection regime or the unconditional effects of the morphine or the vinegar solution.

Control over consumption exerted by morphine was mediated specifically by central nervous system opiate receptors. An injection of 0.2 mg/kg of naloxone eliminated the capacity of morphine to control consumption of vinegar, whereas an injection of 0.1 mg/kg of naloxone produced a decrease (of 0.1) in the consumption of vinegar. Such results suggest that the consumption of vinegar is mediated by opiate receptors in the brain. The positive reinforcing properties of morphine that support preference choices do not seem responsible for the discriminative control exerted by morphine over vinegar consumption. We removed the neuronal cell bodies of the nucleus tegmenti pedunculopontinus region of the mesothoracic midbrain, our region of interest, and find that the discrimination remains intact. This lesion prevented 5 mg/kg of morphine from producing place preferences but did not prevent morphine from acting as a cue to cueing the rat for morphine consumption. These data indicate that the motivational properties of morphine that produce taste aversions and place preferences can be dissociated from the cueing properties of morphine that enable morphine to serve as a discriminative stimulus.
4.29.15 SEPARATION OF MORPHINE’S INCENTIVE MOTIVATIONAL FROM ITS ESCAPE FROM WITHDRAWAL PROPERTIES. A. Bechara and D. van der Kooy. Anatomy Department, Faculty of Medicine, University of Toronto, Toronto, Canada M5S 1A8.

In the case of drugs which produce physical dependence such as opiates, it is difficult to differentiate positive motivational or incentive effects from reinforc-
ing effects associated with avoiding or terminating abstinence. In dependent animals most paradigms for assessing the reinforcing effects of drugs measure both properties. Using morphine as a reinforcer in animals employing a modified shock-avoidance conditioning paradigm, we now demonstrate that these two mechanisms of reinfor-
cing are separable.

Three different testing procedures were employed, each comprising two sepa-
rate groups of drug naive and physically dependent rats (50 mg/kg/day for 14 days). Briefly, procedure B (both) was the standard place conditioning method where conditioned place preference was determined in a two-compartment place conditioning apparatus where one environment paired with morphine (2 mg/kg/p) and the other envi-
ronment paired with vehicle injections. Procedure B (morphine only) and D (with drawl only) were modified place conditioning procedures where conditioned place


The involvement of intrinsic neurons of the lateral hypothalamus (LH) in intracranial self-stimulation (ICSS) of the parabrachial area (PBA) was studied. Rats were bilaterally implanted with electrodes into the PBA and subjected to a 7-day program with guide cannulas located above each LH. Rats were tested for ICSS and once responding had stabilized, unilateral ibotenic acid (IBO) lesion of the LH was performed, rats were retested for ICSS in the PBA both ipsilateral and contralateral to the lesion. Intrinsic neurons of the other half of the LH were then lesioned with IBO and ICS in both sides of the PBA was retested 6, 12, and 30 days later. The unilateral lesion produced a significant decrease of ICSS of PBA ipsilateral to the lesion, without modification of the ICSS of the contralateral PBA. After bilateral lesion, ICSS was greatly reduced bilaterally and the results further suggest that the main effect of the lesion was to decrease ICSS threshold.

In a second experiment, rats with bilateral IBO lesion of the LH and control rats were placed, 13 days after operation on a water-deprivation schedule for 5 consecutive days and were then given the choice between water and one of 5 concentrations of saccharin solution using 2 bottles procedure. Fluid intake across concentrations generated a preference-aversive response curve. The same procedure was used to obtain the aversion curve for 5 increasing concentrations of quinine solution. Lesioned and control rats showed a clear preference-aversive response to saccharin solutions and an aversive response to quinine solutions. However, the highest preference score of the lesioned rats was obtained where saccharin concentration was lower than the concentration preferred by control rats. In the lesioned rats, a clear aversion threshold was obtained with a concentration 5 times higher than the concentra-
tion inducing aversion in the control rats. At the end of these experiments, rats on controls were lesioned and the same experiment was repeated in the bilateral lesion of the LH. Although they learned the choice task before the lesion, these newly lesioned rats reproduced the preference-aversive curve to saccharin made lesioned naive rats.

Taken together, our results suggest that: 1) the ICSS in PBA, a relay for gustatory stimuli, is led by the lateral hypothalamus. 2) A deficit of the concentra-
tion of feedback loops between the LH and the PBA; 2) in the normat rat, the palatability of certain gustatory stimuli is modu-
lated by the lateral hypothalamus.

The quantitative distribution of LH neurons projecting to the PBA were studied by retrograde horseradish peroxidase labeling. (Supported by INSERM grant 856056).

4.30.1 PHARMACOLOGICAL AND PHYSICAL ANTAGONISM OF VARIOUS ACTH-INDUCED GROOMING BEHAVIORS: EFFECTS ON BODY TEMPERATURE. B.L. Colburn, D.A. Toumbly*, and M.H. Cleckner. Dept. of Physiology and Biophysics, University of Illinois, Chicago, IL 60680.

Rats injected iv with ACTH, exhibited a dramatic increase in grooming behavior and especially prolonged duration of grooming. Behaviors induced by this peptide (face washing, body washing, scratching, & amnogestal licking) are qualitatively and quantitatively similar to those observed when rats are placed in a novel environment. Grooming also serves the role of temperature regulation. When exposed to warm environments, rats cool themselves by spreading saliva on their fur; in cold environments, they spread a lipid material which insulates and darkens the fur.

Our present experiments which show that iv injection of ACTH (100/300) or exposure to a novel environment produce changes in body temperature which could account for the sub-
sequent increase in grooming. Body temperature was also inves-
tigated during blockade of grooming by neurotensin (200, 400) iv, by naloxone (100, 300, or), or by controls which physically prevented rats from grooming.

Several behaviors (face washing, body washing, scratching, amnogestal licking, stretching, yawning, and crouching) and core temperature were simultaneously recorded for 70 min beginning 10 min after drug injection. Temperature was recorded with bioneometry (model 40, or). Surgically implanted into the peritoneal cavity 7 days before the behavioral session. Rats injected with ACTH exhibited significantly more grooming (particularly face and body washing) than did rats injected with saline. Body temperature was increased over basal temperature by 2°C in both ACTH and saline-treated rats placed in a novel observation chamber; however, body temperature was significantly higher in saline-treated animals. Body temperature was decreased from baseline in rats treated with either neurotensin or naloxone.

On the other hand, when rats were first exposed to a novel environment, grooming induced by saline was suppressed by naloxone, especially during the last part of the session when grooming was most effectively suppressed. In rats, the body temper-

te of saline injected rats was increased compared to those injected with ACTH, even though both groups exhibited com-
parable amounts of elevation; saline suppressed grooming.

The results of these studies suggest that body temperature responds to grooming. However, it is not clear whether changes in body temperature are a cause or consequence of altered grooming, or are mainly concomitant with these behaviors.


Bilateral microinjection of oxytocin (OXY) into the ventral tegmental area (VTA) of freely moving, saline-primed rats induced prolonged grooming behaviors in grooming behaviors at doses from 100 pg to 400 ng. Sites in the caudal region of the VTA were more effective than sites in the rostral region of the VTA. The time course of action of OXY in the grooming paradigm indicated onset beginning immediately after injection, and termination at 60-75 minutes after injection. Control rats, infused with vehicle, or saline, showed no significant difference in behavior in terms of grooming.

Injection of oxytocin into the VTA resulted in grooming behaviors in a dose dependent manner, particularly in female rats. Oxytocin-induced grooming was not observed in male rats, and accordingly, no significant difference in behavior was observed in males. These results suggest that the effect of OXY is related to sex differences in behavior.

Oxytocin-induced grooming was not observed in male rats. Oxytocin-induced grooming was not observed in female rats. Oxytocin-induced grooming was not observed in animals administered with vehicle or saline. Oxytocin-induced grooming was observed in animals administered with vehicle or saline. Oxytocin-induced grooming was observed in animals administered with vehicle or saline. Oxytocin-induced grooming was observed in animals administered with vehicle or saline.

The results of these studies suggest that OXY is related to sex differences in behavior.
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430.3 GROOMING AND GnAWING DURING HALOPERIDOL-INDUCED CATAPLEXY. C. Van Bartensveld, Psychology Department and Center for Neurobiology, Neuroendocrinology, & Immunology, University of Ottawa, 275 Nicholas St., Ottawa, Ont., Canada. K1N 6N5

Measurements of haloperidol-induced cataplexy such as clinging to a horizontal bar or to a vertical wire grid increase in a dose-dependent manner. In this experiment grooming and gnawing, behaviors not usually associated with cataplexy, were also found to increase significantly with increasing doses of haloperidol. Male Long-Evans hooded rats were each injected IC with each of four doses of haloperidol in a randomized order: 0.05, 0.1, 0.2, and 0.4 mg/kg. After injection each rat was tested every 30 minutes for two hours for both horizontal bar clinging and vertical wire gnawing. For the former, each rat was placed with its forelimbs on a horizontal bar, and duration of bar clinging without moving a forelimb was measured in two trials with a 30 second inter-trial interval. After another 30 second interval, the rat was placed on a near-vertical wire grid, and duration of gnawing was also measured in two trials with a 30 second inter-trial interval. The cutoff for each clinging trial was 2 minutes. During these tests the occurrence of grooming and gnawing were also noted.

As expected, both horizontal bar clinging and vertical grid gnawing increased significantly as the dose of haloperidol increased. There was also an unexpected increase in both gnawing and grooming during the cataplexy tests. After the highest dose, rats frequently gnawed while on the horizontal bar, and gnawed on the vertical wire grid. Number of rats grooming or gnawing increased significantly in a dose-dependent manner, as did number of gnawing episodes. These findings were unexpected since in some situations both gnawing and grooming can be induced by dopamine agonists, and both can be blocked by dopamine antagonists such as haloperidol. Since these behaviors were not observed in the home cage between trials, they may be due to an interaction between haloperidol and the procedures of cataplexy testing.

430.4 BOMBESIN-INDUCED GROOMING IN THE GOLDEN HAMSTER: NEUROPHARMACOLOGICAL BLOCKADE. P.J. Kulikovsky, M.A. Podger*, V.F. Slizewicz, V.I. Shevchenko, Dept. of Psychology, Univ. of Southern Colorado, Pueblo, CO 81001

Bombesin (BBS) is an anuran-derived tetradecapeptide with structural counterparts in mammalian brain and gut that are viewed as candidate neuromodulators. Cerebral ventricular administration of BBS and BBS-like peptides robustly elicits a syndrome of excessive grooming and scratching behaviors in the golden hamster. Male and female golden hamsters received intracerebroventricular (icv) injections of either artificial cerebrospinal fluid (CSF) or bombesin (BBS1-14) and grooming behavior was observed and categorized at tone-cued 0.6 sec intervals each minute for 60 min after injection. The threshold dose of BBS-induced excessive grooming was 0.1 μg in the hamster. Grooming persisted throughout the observation period at doses of 0.1 and 1.0 μg. At all effective doses, forepaw and mouth grooming behaviors were increased more than hindleg scratching behavior. To assess neuropharmacological sensitivity of this effect, hamsters received either intraperitoneal saline or 5.8 mg/kg haloperidol, 1.0 mg/kg haloxone, 28.8 mg/kg pentazocine, or 0.4, 0.8, 1.0 and 1.2 mg/kg scopolamine, 15 min prior to icv CSF or BBS. BBS was found to be catalepsy (both horizontal and vertical) dose-dependently and slightly reduced BBS-induced grooming, but did not affect basal level of grooming. Scopolamine abolished BBS-induced grooming at doses of 0.1 and 1.8 mg/kg, but did not abolish basal level of grooming. The blockade of BBS-induced grooming by peripheral scopolamine is consistent with observations made in the rat by Morali, Johnston and Kateb (Abst. N.Y.A.C. Conf. Neural Mech. Behav. and Motil. in Normal & Pathol. 1986), and additionally, we have recently considered the use of antimigraines in treatment of puritis associated with BBS-secreting liver tumors. The results that follow characterize a BBS-induced grooming in the golden hamster as potent and persistent, involving primarily forepaw and mouth grooming behaviors. Influent mechanisms, peripheral and central are involved, and demonstrate centrally-mediated cholinergic neural activity. These results confirm a cross-species generality of BBS-induced grooming, but also indicate the specificity of response to topography and neuropharmacological sensitivities of this neupeptide effect on skin surface maintenance behaviors. (Supported by NIH Grant No. HD-38197)

430.5 ENHANCED NOVELTY-INDUCED GROOMING RESPONSE OF THE STOMATIC RAT MAY BE ASSOCIATED WITH D-1 RECEPTOR SUPERSENSITIVITY. S. Merali & Q. Ahmad, Psychology & Pharmacology, Dept. of Psychology, University of Ottawa, 275 Nicholas St., Ottawa, Ont., Canada. K1N 6N5

In the spontaneously diabetic Wistar BB-Rat (BBDR), we have observed an attenuated behavioral response to d-amphetamine, morphine and codeine. However, the grooming response to a novel environment was significantly enhanced in the BBDR as compared to the non-diabetic animals. Under a more stressful condition (two serial l.p. saline injections), the non-diabetics and BBDR gowned equally as much. Since the dopamine D1 receptors have been implicated in the expression of grooming, we wanted to explore whether the sensitivity of the D1 receptors was increased in the BBDR. Thus BBDR rats (maintained on insulin therapy) and age-matched control rats were administered a selective D1 receptor agonist SKF 38393, 0.00-10.0 mg/kg l.p. and the grooming monitored for 1 hr. A time-course analyses revealed that most of the grooming occurred within the first 15 min following treatment. Hence all the data were analyzed for that duration only. In control rats (genetically distinct group), SKF 38393 induced grooming in a dose-dependent manner at the 5 and 10 mg/kg doses, control rats groomed 3x and as much, respectively, as at the 0.1 mg/kg dose. In the BBDR group, scopolamine slightly affected the grooming of SKF 38393. A small percentage of the rats were slightly more sensitive to the grooming effects of SKF 38393 than the genetically distinct control rats but no significant difference was observed at the DNR. Thus our data indicates that the BBDR may have supersensitive D1 receptors. This may be a compensatory response to the reduced grooming behavior in the diabetic rats. A significant enhancement was also observed in the response of novelty-induced grooming. Therefore, our data also suggests that D1 receptors may be involved in the expression of novelty-induced grooming.

430.6 CODEINE AND MORPHINE EFFECTS ON GROOMING. A.K. Banks*, V.H. Cigan*, J. Erbacher*, E. Shevchenko*, AND P.J. Kulikovsky, Center for Neurobehavorial Sciences, Dept. of Psychology, University of southern Colorado, Rm. 236, Binghamton, NY 13901, AND Institute of Molecular Biology, University of Utrecht, Utrecht, Netherlands

Previous studies have shown that the codeinone RX 336-M injected peripherally can induce excessive grooming in rats. The amount of grooming elicited by the drug was both age- and sex-dependent. No excessive drug-induced grooming was observed in rats of either sex at age older than 60 days. The present studies were undertaken to determine whether codeine, itself, administered peripherally, would have similar effects to the codeinone. Because of the chemical similarity between codeine and morphine, we also examined grooming elicited by the peripheral injection of morphine as compared to that elicited by codeine. In addition, we studied grooming behaviors elicited by the central administration of codeine.

For the peripheral injections, a dose of 3.0 mg/kg (free base) of codeine or morphine was used. Intraventricular (icv) dosages of 0.3 μg/kg or 1.0 μg/kg. In each study, drug-treated animals were compared to animals injected with an equal volume of saline. The icv administration of the drug and the scoring of excessive grooming were accomplished using methods commonly employed in this laboratory.

Peripheral injections of codeine produced an enhancement of grooming over saline controls. The effects were greater in younger than in older animals. However, in contrast to the results obtained with RX 336-M, a significant enhancement of grooming was also found after peripheral administration in the adults. Peripherally administered morphine produced a different pattern of effects than that of codeine. These rats were suppressed grooming during the first 30 minutes of observation, and accentuated grooming over controls amounting to 60-75% after the start of observation. Suprisingly, central administration of codeine, at either of the dosages used, had no effect on grooming. This is in contrast to studies that have shown that icv administration of morphine produces excessive grooming and does so in the same manner as it does following peripheral administration. Based on these findings, therefore, it appears that codeine and morphine induce excessive grooming by different mechanisms.

Deer mice, Peromyscus maniculatus, provide an opportunity to assess the effects of natural selection on opioid activity and behavioral responses to stress. Deer mice from various habitats and geographical areas differ in a variety of behavioral, ecological and physiological characteristics. Of particular evolutionary interest are the differences between island and mainland populations of deer mice and other small rodents. In the present study, we describe the restraint-stress induced opioid analgesic and locomotor responses of four different populations of male and female deer mice, Peromyscus maniculatus aztecianus and P. g. nebraskiensis from two mainland regions, and P. g. maniculus and P. g. trilineatus from two small coastal islands. All of the mice displayed immobilization-induced analgesia which was blocked by peripheral administrations of the prototypical mu opioid antagonist naloxone (1.0 mg/kg). In all of the populations, males displayed significantly greater levels of analgesia than females. In addition, the levels of analgesia were significantly greater in the inbred than in the mainland male and female mice. Restraint also induced significant increases in the locomotor activity of the mainland deer mice, while significantly decreasing the activity of the insular animals. Males displayed significantly greater stress-induced changes in locomotor activity than females. The stress-induced increases in activity of the mainland populations were blocked by the specific delta opioid antagonist, ICT 354,129 (10 mg/kg), while the decreases in activity of the insular animals were blocked by naloxone (1.0 mg/kg). These findings demonstrate that there are marked sex and population differences in the responses to stress and opioid activation in deer mice. These results suggest that behavioral differences between island and mainland populations may, in part, be related to differences in endogenous opioid activity.

WHEEL RUNNING AND GASTRIC ULCERATION IN THE RAT FOLLOWING PREADAPTATION TO RESTRICTED FEEDING. B.G. Novotny and S.R. Kieffer. Dept. of Psychology, Kansas State University, Manhattan, KS 66506.

When a rat is placed in an activity wheel and is restricted to eating for 1 hr each day, it runs progressively more, develops ulcers, and results in the demise of the animal. The relationship between preadaptation to restricted feeding, gastric ulceration, and elevated running is not clear as conflicting results have been reported (see Fare et al., Physiol. Behav., 1985, 34, 423-429). In the present study the effect of preadaptation to restricted feeding on #wheel running and ulceration was examined by preadaptation of animals for either 15, 25, or 35 weeks. Fifty-six naive rats were housed in activity wheels for 5 days to record baseline measures of food intake, water intake, body weight, and running activity (recorded across 3 daily time periods). Rats were then matched on mean baseline activity and divided into 4 experimental (n=7 each) and 4 control groups (n=7 each). Groups were preadapted 1 hr restricted feeding schedule for either 0, 15, 25, or 35 days; experimental groups re-entered the activity wheels following preadaptation experience, whereas control groups remained in individual home cages. Rats were fed between 7:30 PM and 8:30 PM. Food intake, water intake, body weight, and running activity was recorded on a daily basis. Activity rate (and home-cage rate) were sacrificed at the end of the 18 day testing period or whenever an activity animal became moribund. Following sacrifice, the stomach was excised and later scored for gastric ulceration.

Regardless of preadaptation experience, total daily running increased, with the greatest change occurring during the time period (11:10 AM to 6:30 PM) preceding the feeding hour. Running during the remaining two times did not change significantly. Rats with previous preadaptation experience increased running sooner than rats not preadapted. Results further indicated that rats preadapted for 15 days had significantly fewer ulcers (p<.05) than the nonadapted group. Rats preadapted for 25 and 35 days also had less ulcers than rats not adapted (p<.05). Increasing preadaptation experience directly increased the number of rats which survived. Results are discussed in terms of the gastric status of the rat as an impetus for excessive wheel running.


Recently, various populations of deer mice, Peromyscus maniculatus, have been used to examine the ecological relevance and adaptiveness of stress-induced opioid activation and analgesia. Body rotation, which has been related to vestibular stimulation and motion sickness, is a commonly used stressor that is of both basic and applied interest. In the present study we examined the effects of continuous and intermittent body rotation on the nociceptive responses of island and mainland derived populations of deer mice, P. g. aztecianus and P. g. nebraskiensis respectively. These populations were chosen because their natural habitats differ, the island populations being found to habitat in which they are more likely to experience complex vestibular stimulation.

Various groups of island and mainland populations of male mice were exposed to either continuous (79 rpm) or intermittent (5, 30 sec on, off) body rotation for varying periods of time (2-30 min), at specific times (0-60 min) after the rotation the foot-licking response to a surface held at 50°C was determined for individual mice. Mice exposed to either continuous or intermittent rotation were analgesic, displaying significantly greater response latencies than sham-rotated or control animals. Pre-treatment with naloxone (1.0 mg/kg) blocked both the continuous and intermittent rotation-induced analgesia. In addition, there was a greater level of analgesia after exposure to the intermittent rotation than the continuous rotation. For all cases, the insular mice displayed significantly greater levels of rotation-induced analgesia than did the mainland population animals. The results indicate that the degree of body rotation-induced analgesia differs between population in a manner consistent with the likelihood of being exposed to vestibular stimulation in the wild. These findings may have implications for the evolutionary basis of motion sickness.


Modification in dietary calcium has been shown to alter blood pressure in the spontaneously hypertensive rat (SHR). Supplemental calcium attenuates the development of hypertension in these rats while calcium deprivation potentiates the rise in blood pressure. Similar results have been seen with respect to blood pressure reactivity. A low calcium diet causes greater pressor responses to stress while high calcium reduces reactivity.

This study was designed to determine whether increased catecholaminergic activity or decreased sensitivity to catecholamines might be responsible for differences across diets. Both the SHR and its normotenstive control strain the Wistar-Kyoto (WKY) were maintained on low (0.1%), medium (1.0%) and high (2.0%) calcium diets and assessed for stress-induced increases in circulating catecholamines as well as pressor responses to catecholamine infusion. Results of this study demonstrated that SHRs had significantly higher blood pressures than their normotensive control strain, the Wistar-Kyoto (WKY). Furthermore, SHRs on a low calcium diet demonstrated higher blood pressures during both handling and restraint stress than did SHRs maintained on a high calcium diet (p<.05). In contrast, unanesthetized SHRs showed no difference in blood pressure across dietary conditions. No dietary effects were seen in the WKY. Analysis of stress-induced release of circulating catecholamines demonstrated no difference between the 2 diets in the SHR. However, determinations of catecholamine sensitivity in a dose response study indicated that SHRs maintained on a low calcium diet exhibited significantly larger increases in blood pressure to noradrenaline infusion (p<.05). Thus, it seems likely that differences in blood pressure reactivity to stress observed in the SHR are a consequence of altered post-synaptic sensitivity and not an increase in circulating catecholamines.
recently, we determined in rabbit that (a) a fear-arousing conditioned stimulus (CS) during recent exposure of Pavlovian fear conditioning induced arrhythmias in the presence of digitals, and (b) vagalactivation in response to the CS contributed to the generation of these arrhythmias (markgraf et al., 1985). Furthermore, we demonstrated that electrical stimulation of the amygdaloid complex under these circumstances in the expression of vagus-mediated conditioned bradyarrhythmia in the rabbit (kapp et al., 1984), during digitals infusion induced vagus-mediated arrhythmias similar to topography to those observed in the CS.
Since lesions of the ACE attenuate the retention of vagus-mediated, conditioned bradycardia in response to a fear-arousing CS (gentile et al., 1986), we sought to determine if ACE lesions would attenuate CS-induced arrhythmias in rabbits receiving digitals during retention. Twenty New Zealand rabbits received 20 Pavlovian fear conditioning trials in which a 5.0 sec tone CS was paired with a 0.25 sec, 2.0 mA shock. Twenty four hours later, five rabbits showed a low rate bradycardia in response to the tone CS which were implanted prior to conditioning. Nine rabbits served as sham operated and six as unoperated controls. Twenty-four hours following the lesion procedure, a retention test was given during which digitals was infused i.v. (110 ug/kg), and 20 CS alone tone were presented. The results demonstrated a significant increase in the frequency of arrhythmic episodes during the CS exposure compared with pre-CS baseline frequencies for both sham and unoperated controls (p < 0.05). No such increase in the frequency of arrhythmic episodes during the CS was observed in the ACE lesion group. The frequency of arrhythmic episodes during the pre-CS period was unaffected by lesions of the ACE.
The results confirm our previous results demonstrating that ACE stimulation induces arrhythmias and suggest that the ACE contributes to the generation of arrhythmias in response to a fear-arousing CS in the myocardium providing for the electrical instability with digitals. Supported by a grant from the American Heart Association, Vermont Affiliate.

Recent research has reviewed the existence of individual differences in the predisposition to develop schedule-induced polydipsia (SIP) in rats exposed to intermittent food deprivation (Tazi et al., 1987). In the present experiments, we have further explored the physiological and behavior differences in SIP-positive and SIP-negative rats.
In experiment 1, rats implanted with a chronic venous catheter were submitted to daily sessions of intermittent food. SIP-positive rats exhibited a decrease in plasma corticosterone levels, while SIP-negative rats showed a gradual increase in plasma corticosterone levels. There was, however, no differences in plasma c-releasing hormone (Crh) levels that increased uniformly in the two groups. When all the rats were tested without water, SIP-positive rats displayed an increase in corticosterone levels similar to that observed in SIP-negative rats.
In experiment 2, rats previously tested for SIP were submitted to conditioned avoidance learning in a 2-way shuttle box. SIP-positive rats learned faster than SIP-negative rats. Moreover, a positive correlation was observed between the amount of water consumed and the number of avoidance responses (rs = 0.56, p < 0.05).
Experiment 3 extended this observation to a social situation where the positive and the negative rats were placed as intruders into the home cage of a resident rat that had previously been habituated to the intruder. Videotape observation of the behavior of the intruder during a 10 min session revealed a tendency for SIP-positive rats to demonstrate a submissive-defensive behavior than SIP-negative rats in response to the resident's attacks. These data indicate that differences in the predisposition to develop avoidance behavior are accompanied by differences in emotionality and behavioral differences. Whether such differences preexist to the SIP experience or are the result of repeated episodes of excessive drinking is not yet determined.

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Schedule-induced polydipsia (SIP) is an example of the larger category of schedule-controlled behaviors that occur in situations where a strongly motivated appetitive or consummatory behavior is interrupted or prevented (as in situations where SIP as well as other "adverse" behaviors are adaptive as they serve to maintain an animal in a situation that is favorable to its survival. Consistent with this hypothesis is the observation that SIP if a coping response that can reduce the heightened arousal caused by an intermittent schedule of food presentation. Hungry animals exposed to intermittent presentations of small amounts of food show increased arousal as measured by either endocrine or behavioral indices (Brett, J.P., and Levine, S., J CEP, 93:966, 1979; Elleen, F., Hanson, S.J. and Osborne, S.K., Psychol. Rev., 85:571, 1978).
In support of the coping hypothesis, pildulatory-adrenal activity is significantly reduced in animals exposed to an SIP. However, as Lacey and Lacey (J. Pers. Soc. Psych., 30:1, 1974) have shown, physiological indices of arousal are not always correlated with each other. The interpretation of SIP as a coping response which regulates arousal is therefore critically dependent on which physiological measure is used. In this experiment, rats food deprived to 85% of their baseline weight, were given 15 daily tests for schedule-induced drinking. A food pellet (90 mg) was dispensed every 60 seconds of the 30 minute tests. In addition to recording licking, general activity and entries into the food tray, oxygen consumption and expired heart rate were continuously monitored. By day 10, 73% of the animals had developed significant polydipsia.
There were systematic changes in V20, general activity and tray entries that were closely locked to the point of food delivery. Comparisons between drinking and non-drinking rats and between trials when drinking and non-drinking did not occur indicated that drinking was consistently associated with increased oxygen consumption, increased activity, but decreased heart-rate. These results are consistent with the interpretation that SIP provides a motor outlet for the expression of excessive levels of mobilised c-releasing hormone (CRH) and, however, further characterization of this relationship will require precise measurement of temporal variations in V20 and licking. The findings emphasize the importance of taking multiple physiological and behavioral indices of arousal and question the unitary nature of this construct.

10.14 HDL-CHOLESTEROL ELEVATION BY ACUTE STRESS IN THE RAT. C.D. Cooper and K.P. MurrayJ, Dept. of Psychology, Northern Illinois University, DeKalb, IL 60115.
We previously reported (Cooper et al., Soc. for Neurosci. Abst., 1986, 12, 330) that 90-day-old rats exhibit a suppression of the serum level of cholesterol density lipoprotein (HDL) in response to the stress of a jugular blood sampling (either anesthesia and withdrawal) of 1.5 ml blood from the surgical exposure of the jugular v, vein and a 10 min FS session of a shock (FS) session of 20 min or 1 hr duration. High density lipoprotein cholesterol (HDL-C) tended to decrease and then decrease and then decrease and then decrease, HDL-C levels were significantly lower at 4 and 24 hr following the 1-hr FS session. Neither the jugular blood sample, nor a brief footshock session were found to cause such short-term changes in blood cholesterol.
In the present research, here, 90-day-old male rats were subjected to a jugular blood sample on Day 1, and then on Day 3 were given a FS session involving 2-sec footshocks on a variable-interval 2-min schedule at an intensity of 1.4 mA no-holding setting on SSR-Forgor SSGS-003 shocker / scramblers (equivalent to -7.1 mA on most shockers), followed by a final blood sample. If no footshock were given on Day 3 (FS session duration 0), the blood showed, compared to Day 1, an 8.7% increase in total serum cholesterol (from 73.8 to 80.7 mg/dl, t(16) = 7.1, p < .001) but was associated with an 8.6% increase in HDL-C from 48.7 to 52.9 mg/dl, (t(16) = 4.84, p < .001). From this elevated baseline on Day 3, total serum cholesterol levels were not significantly changed by footshock exposure. In contrast, HDL-C was raised over the higher baseline of Day 3 by an additional 10.1% with a 20 min FS session, (t(22) = 3.01, p < .01), and was continuously monitored.
We found that FS sessions of 80, 160, or 320 min, HDL-C levels, like total serum cholesterol, were not different from the Day 3 baseline. However, in additional comparison groups, it was found that the concomitant stress of a footshock did not occur immediately after the beginning 15 min later caused significant drops in total serum cholesterol by the end of the session; this occurred when the rats had also been blood sampled on Day 1 from 8.6 to 8.8, (t(16) = -7.1, p < .01), as well as when they had not from 73.7 to 66.6, (t(16) = -3.67, p < .01). However, further characterization of this relationship will require precise measurement of temporal variations in V20 and licking. The findings emphasize the importance of taking multiple physiological and behavioral indices of arousal and question the unitary nature of this construct.

Spontaneously hypertensive rats (SHR) not only have high blood pressure, but also express an elevated sodium appetite compared to Wistar-Kyoto rats (WKY). We examined whether these traits are genetically coupled to one another. Establishing a linkage between trait manifestation requires that the traits cosegregate in hybrid matings of the parent strains and their offspring. In homozygous strains all genes are "fixed" whether they are related to the trait upon which the strain was selected or not. Thus, SHR and WKY differ in many traits that are not related to blood pressure. In cosegregation analysis, SHR and WKY are cross-registered to generate the F1 offspring which are intercrossed to produce the F2 generation. SHR, WKY, and F1's are homozygous for high, normal, and intermediate blood pressure, respectively, whereas the F2's are heterozygous for the full range of genetically determined blood pressure. If traits are genetically linked they must cosegregate, resulting in a strong correlation in the F2 population. If two traits are not linked, they will segregate randomly with respect to one another in the F2 generation, resulting in no correlation.

We examined the relationship of blood pressure and salt appetite in male SHR, WKY, F1, and F2 rats, 50-140 days of age. Blood pressure was measured prior to salt appetite testing using the indirect tail-cuff method. Subjects had ad lib access (for 6 days) to water, 1.5% NaCl, and Teklad low sodium chow to ensure that saline consumption represented sodium appetite (need) as well as discretionary sodium intake. Fluid intake was measured daily. The mean blood pressure of SHR was significantly greater than all other groups, and the WKY pressure was significantly lower than all others. As expected, the F1 and F2 groups were near midparent values, and did not differ from each other. However, 1.5% NaCl intake did not follow an additive genetic model as does blood pressure. There was a big difference in saline intake between SHR and WKY, but the F1 and F2 rats behaved similar to WKY rats rather than intermediate between WKY and SHR. Moreover, there was no correlation between blood pressure and salt intake in the F2's, indicating there is not a common genetic basis for the two traits.

Another lab also reported recently that salt appetite and blood pressure are not related in F2 rats (Harrap, Hypertension, 1986). However, that study, which used hypotonic (~22%) saline, found that F1 and F2 salt appetite was SHR-like rather than WKY-like. Thus, it is possible that neither the WKY nor the SHR salt appetite phenotype is dominant. Instead, it appears that phenotypic expression of salt appetite in F1 and F2 rats may be partially determined by the concentration of the saline solution.

REGIONAL VARIATIONS IN STRESSOR PROVOKED ALTERATIONS OF INTRACRANIAL SELF-STIMULATION FROM THE VENTRAL TEGMENTAL AREA. Marilyn Kasian*, Robert M. Zacharko and Hyние Anisman. Dept of Psychology, Carleton University, Ottawa, Ontario, Canada K1S 5B6

Uncontrollable stressors alter the rewarding value of intracranial self-stimulation (ICSS) from mesolimbic sites, without affecting responding for brain stimulation from the nigrostriatal system. In particular, marked reductions in rates of responding for previously rewarding brain stimulation and changes in current intensity functions have been noted from both the nucleus accumbens and the ventral tegmental area (VTA), while ICSS from the substantia nigra is unaffected by stressor application. These brain region specific effects of uncontrollable stressors on ICSS performance appear to parallel the stressor induced variations of dopamine (DA) activity, in that stressors were found to affect mesolimbic and mesocortical DA turnover, without affecting DA activity in the substantia nigra. We now report that the effects of uncontrollable stressors on ICSS in several strains of mice (C57BL/6, BALB/cBy) varies across different portions of the VTA. Furthermore, the effects of desmethylimipramine (DMI) on stressor induced responding from these regions are distinguishable from those previously noted from the nucleus accumbens, where repeated DMI antagonized the behavioral suppression induced by a stressor. DMI treatment was generally ineffective in reversing ICSS alterations from the VTA. In accordance with the anatomical descriptions provided by Lindvall and Bjorklund (1984), HRP tracings were determined for the ventral, lateral and dorsal regions of the VTA. Our results suggest that the pathways ascending from these relatively discrete areas of the VTA are differentially sensitive to uncontrollable stressor application. This differential sensitivity is in turn reflected in distinct ICSS profiles.

EFFECTS OF RAT MEDIAL FRONTAL CORTEX LESIONS ON CONDITIONED EMOTIONAL RESPONSES. R. J. Frystaek* and R. H. Neasey. Department of Anatomy, Loyola University Medical Center, Maywood, IL 60153

The rat medial frontal cortex (MFC) projects directly to the vagal solitary nucleus (Torruberry & Neasey, HR 278, 1983), and this pathway appears to influence heart rate (HR), blood pressure (BP), and respiration in both the awake and anesthetized rat. We have begun studying the effects of MFC lesions on the HR and BP components of the conditioned emotional response (CER) in rats.

Male Sprague-Dawley rats were used; four sustained bilateral MFC lesions (bregma to frontal pole, surface to 5 mm below surface, midline to 2 mm lateral) via aspiration, while controls consisted of sham operated and one normal rat. All rats were conditioned two weeks after the initial surgery. For conditioning, the conditioned stimulus was a 10 second, 800 Hz tone, and the unconditioned stimulus was a 2 amp (1 sec duration) footshock delivered through a metal grid floor. The conditioning process consisted of 10 tones for orientation, followed by 30 tones presented immediately with the footshock. After conditioning, the animal was anesthetized and femoral artery and vein cannulas were implanted along with EKG leads. The following day, BP and HR were recorded during CER trials using a chart recorder and a microcomputer. Data were recorded for a 35 second period, with the tones occurring at 10 seconds; the 5 seconds prior to the tone served as the baseline for each trial. Three CER trials were averaged second by second for each animal. These individual animal averages were then averaged into a composite average for each of the two groups (9/4 in each group).

The BP responses in controls and lesioned rats were similar. Both responded initially to the tone with an increase of ~20 mm Hg. The HR responses between the two groups contrasted sharply (see figure; error bars indicate ± SEM). The controls responded to the tone with a slight increase initially (~5 bpm) followed by a large increase coinciding with the time of the expected shock (~20 bpm). The lesion group responded with a decrease in HR, with the maximum drop (~20 bpm) occurring near the time of the expected shock. This pattern of BP + HR responses in the lesioned group resembles that of the normal baroreceptor reflex, suggesting a possible mechanical distortional stress for normal animals, the baroreceptor reflex may be inhibited by the lesioning of the MFC, and HR and BP both increase. By lesioning the MFC, the cortical terminals in the NTS which may inhibit the baroreceptor reflex are lost, thus allowing this reflex to operate during the CER. (Supported by a Post's fund grant from Loyola University.)
431.1 ZINC ALTERS GLUTAMATE TOXICITY ON CORTICAL NEURONS. J. Roh and D.W. Choi, Department of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Chelatable zinc (Zn) may be co-released with glutamate in both normal synaptic transmission and in disease states such as hypoxia and epilepsy. We have recently shown that Zn blocks NMDA receptor-mediated excitation and toxicity on cultured cortical neurons, while often potentiating the excitation produced by quisqualate or ibotenate. Elsewhere (S. Peters and D.W. Choi, this meeting), we described the effect of Zn on glutamate neurotoxicity and support here the ability of Zn to alter glutamate neurotoxicity.

Exposure of mature cultured murine cortical neurons to 500 μM glutamate for 5 min resulted in the following day in widespread neuronal disintegration and a substantial efflux of lactate dehydrogenase (LDH) to the bathing medium. Addition of 1-30 μM Zn to the glutamate-exposure solution produced a concentration-dependent attenuation in both morphological neuronal damage and the LDH efflux, with the higher Zn concentrations showing a more pronounced effect in neuronal injury at 300-500 μM. Higher concentrations of Zn were intrinsically neurotoxic, resulting in a "U-shaped" Zn concentration-response relation. Addition of 10 μM Zn did not reduce glutamate neurotoxicity.

As previously reported, 500 μM Zn completely blocked the neuronal damage by a 5 min exposure to 500 μM NMDA. However, this Zn concentration, which lacked intrinsic cytotoxicity, potentiated the minimal neuronal damage caused by low grade exposure to either quisqualate (30 μM for 5 min) or kainate (1 μM for 5 min), the former more strikingly than the latter.

Cortical neurons containing NMDA receptor (an enzyme which co-localizes with somatostatin and NPY in forebrain) are selectively resistant to NMDA toxicity and, at the same time, selectively vulnerable to non-NMDA toxicity. Excess glutamate exposure destroyed this marker subpopulation in a non-selective fashion (i.e., in parallel with the general population). As expected, co-application of the NMDA antagonist APV with glutamate attenuated overall neuronal loss to a greater extent than diaphorase-containing neuronal loss. However, while co-application of 300 μM Zn with glutamate also reduced overall neuronal loss, it significantly increased the destruction of the diaphorase-containing neuronal subpopulation.

These observations suggest that Zn, at concentrations which may be realized at excitatory synapses in vivo, could importantly modify the nature of glutamate neurotoxicity in disease states, potentiating NMDA receptor-mediated toxicity, while perhaps increasing non-NMDA receptor-mediated toxicity.

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431.2 ZINC MODULATES GLUTAMATE EXCITATION OF CORTICAL NEURONS. S. Peters and D.W. Choi, Department of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

The transition metal zinc (Zn) is contained in neuronal synaptic vesicles in forebrain excitatory synaptic boutons, and is released in a calcium dependent manner by high K or electrical stimulation. In a recent study we showed that Zn blocked NMDA receptor-mediated neuroexcitation, while usually potentiating the excitation produced by AMPA and Kainate.

In the present study, we examined the effect of Zn on the neuroexcitatory action of synaptic agonists, which is likely to be the actual substance co-released with Zn at many excitatory synapses. Intracellular recordings were made from murine cortical neurons in culture, using voltage-clamped recording medium containing 1 mM Mg.

Zn (50 μM to 1 mM) pressure ejected immediately prior to injection of glutamate (100 μM), reversibly attenuated glutamate depolarizations to 57±11% (n=10) of control amplitude, while sometimes prolonging the response duration. In comparison on several cells, Zn and the selective NMDA antagonist APV (500 μM) produced a similar degree of attenuation of glutamate responses. Zn blocked the initial rapidly desensitizing component that occurred in some glutamate responses, reflecting the predominant contribution from NMDA receptors. In the bath presence of the non-competitive NMDA antagonist ketamine (1 μM), glutamate responses were increased by prior injection of Zn, consistent with the notion that the action of glutamate at quisqualate receptors can be potentiated by Zn.

To test the effect of Zn on spontaneous synaptic activity, cultures were bathed in Mg-Free recording medium, which usually resulted in a high level of continuous and sometimes bursting excitatory activity. Pressure ejection of 1 mM Zn near the impaled cell somata attenuated spontaneous excitatory synaptic activity in about 1/5 of cells tested. In a minority of cells (n=5), the Zn eliminated spontaneous synaptic activity, with some reduction in excitatory activity, pressure ejection of recording medium alone was without effect.

Our results support the hypothesis that Zn, co-released with glutamate at excitatory synapses, may modulate the postsynaptic action of glutamate, reducing its effect at NMDA receptors, and enhancing its effect at non-NMDA receptors.

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431.3 GLUTAMATE TOXICITY IN A NEURONAL CELL LINE IS BLOCKED BY CALCIUM ION ANTAGONISTS, LITHIUM, OR MEMBRANE DEPOLARIZATION. T.H. Murphy*, R.L. Schaar, J.T. Coyle, and A. sage, Dept. of Pharmacology, Pharmacology, and Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore MD 21205.

Previously studied demonstrated that N18-Re-105 neuroblastoma hybrid cells bind [3H]-glutamate with a specific component similar to the glutamate preferring receptor of brain. Incubation of these cells in medium supplemented with quisqualate (50 μM) or NMDA (20 μM) resulted in glutamate (5 μM) receptor) occurred in greater than 70% cell lysis within 24 h, while incubation with kainate and N-methyl-D-aspartate (NMDA) (agonists for other GLU receptors) had no effect. The cytotoxic effect was due to increased cell death by a glutamate receptor, as observed in 57 cells and averaged 36±0.6% in contrast, "picrotoxical" NMDA (10 mM) and L-aspartate (50 mM) did not result in depolarization.

Desensitization. Repeated "picrotization" of application of 50 μM GLU resulted in a 66±10% (n=8) reduction of the GLU response within 30-60 sec. Within 60 sec of cessation, the response to GLU returned to 107±29% (n=8) of control levels. Consistent with desensitization by repeated "picrotization", chronic application of GLU (10 mM) did not produce any significant change in cell membrane potential (18 mV) in the absence of GLU, and was also blocked by 10 mM of the 16 mV of exposure, GLU-induced cell lysis was maximal during 12-18 hours of exposure.

Chronic depolarization blocks L-glutamate's toxicity. When N18- Re-105 cells were depolarized with 50 μM KCl, the cells in the absence of glutamate (50 μM) increased by more than 15% above control levels. Membrane potentials measured in the presence of the protective concentrations of depolarizing agents (and of the absence of GLU) were between 18-20 mV, while unstimulated cells averaged 14±2 mV. Blockade of GLU toxicity by chronic depolarization may be consistent with the hypothesis for two types of sensitivity may be inactivated at depolarized potentials (Nowisky, M.C., et al., Nature, 316:440(1985).

Lithium blocks toxicity. Lithium (20 mM) blocked greater than 70% of GLU toxicity, with little effect on control cell viability or membrane potential, suggesting a role for inositol phosphates in GLU toxicity. The absence of a direct correlation between glutamate depolarization and GLU toxicity in the N18-Re-105 cell line suggests that second messengers other than membrane potential may be involved. Supported by Grants NS 15384 and GM 0726.


Recently A.B. Young and colleagues (reported this meeting) found that THA can displace 1-1-[2-thienylcyclohexy]pipеридин (TCP) binding to the NMDA receptor complex, and in view of several studies suggesting a link between the TCP receptor complex and neurotoxicity, we decided to see if THA could antagonize the toxic or excitatory actions of NMDA on cortical neurons. A 5 min exposure of N18 cells to NMDA (15-21 X 10-7 M) caused a dissociated murine neocortical cell cultures either to 500 μM NMDA or 500 μM quisqualate, resulted 1-4 h in widespread neuronal disintegration and a large efflux of lactate dehydrogenase (LDH) into the bathing media. Addition of 1-3 mM concentrations of THA to the NMDA exposure solution almost completely blocked both the morphological evidence of neuronal cell loss and its biochemical correlate, LDH efflux, but did not much reduce quisqualate-induced neuronal loss. Lower (100-300 μM) concentrations of THA afforded only slight protective effect against NMDA. InTRA neurites but had no effect, while 1 μM concentration of THA showed some weak and variable neuron-protective efficacy against NMDA.

Intracellular biochemical and electrophysiological evidence supported an action of THA on NMDA receptors. Pressure ejection of THA (500 μM to 2 μM) usually produced an increase in membrane resistance and a transient membrane potential increase of 16-18 mV of exposure. THA also substantially attenuated the subsequent depolarizing response to pressure ejection of NMDA. Picrotoxin (2 μM) was less effective in attenuating the response of depolarizing agents (110 μM KC, 230 μM ouabain, or 50 μM veratridine), GLU-induced toxicity was reduced 49-89%, (n=8), and was invariably reduced 100%, (n=6), in the presence of THA (n=8), or the presence of THA (n=6). In the absence of THA, however, not increased more than 15% above control levels. Membrane potentials measured in the presence of the protective concentrations of depolarizing agents (and the absence of GLU) were between 18-20 mV, while unstimulated cells averaged 1-16 mV. Blockade of GLU toxicity by chronic depolarization may be consistent with the hypothesis for two types of sensitivity may be inactivated at depolarized potentials (Nowisky, M.C., et al., Nature, 316:440(1985).

Lithium blocks toxicity. Lithium (20 mM) blocked greater than 70% of GLU toxicity, with little effect on control cell viability or membrane potential, suggesting a role for inositol phosphates in GLU toxicity. The absence of a direct correlation between glutamate depolarization and GLU toxicity in the N18-Re-105 cell line suggests that second messengers other than membrane potential may be involved. Supported by Grants NS 15384 and GM 0726.
431.5 1,2,3,4-TETRAHYDRO-9-AMINOCYCLOHEXANE AND OTHER ACIDINE DERIVATIVES ARE DISPLACERS OF SPECIFICALLY BOUND [3H]-N-(1-2-TRIMETHYLAMINOETHYL)-3,4-piperidine (TCP), a potent PCP analogue, from rat brain membranes. The IC50's were: THA, 70 μM; 9-amincocyclohexane, 150 μM; profalin, 30 μM; phystostigmine and edrophonium ~200 μM; mecamylamine—pyridinium and 3,4-diaminopyridine, >100 μM. THA did not displace [3H]glutamate from NMDA receptors as determined by quantitative autoradiography.

Our results demonstrate that THA and other acidines interact with the dissociative amnesthetic receptor albeit, somewhat weakly. Other 3,4-diaminopyridine (DIDS) antagonists were considered to be weaker as TCP displacers. These results, together with those of Choi and colleagues, suggest some of the effects by interacting with the PCP/NMDA receptor complex. The beneficial effects of THA in AD may be attributable to its modulation of both cholinergic and glutamatergic neurotransmission.

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The excitatory transmitters, glutamate (Glu) and aspartate (Asp), are neurotransmitters capable of damaging CNS neurons by an excitotoxic process. The acute cytotoxic events follow an irreversible stage of necrosis which is followed by edema or infarction. Hypoxia and ischemia occur very rapidly, leaving central neurons in an advanced irreversible stage of neuronal damage. Efforts to prevent such degeneration by in vivo administration of Glu antagonists have, thus far, concentrated primarily on models in which the degenerative process is a single type of neuron—the CA1 hippocampal pyramid—and the degenerative process is of the delayed type, occurring over a 4 day period. Although severe anoxia-ischemia can certainly cause more acute brain damage, prior studies have not established that such damage has pathomorphologic characteristics of acute Glu-like excitotoxic damage. Here we demonstrate in the rat infant that unilateral occlusion of the carotid artery followed by exposure to hypoxic or hyperbaric conditions causes neurons in several brain regions to degenerate by an acute fulminating process that appears identical by light or electron microscopy to that seen in the infant retina or hypothalamic following subcortical administration of exogenous Glu. This may be useful in clarifying the ability of Glu antagonists to protect against anoxic-ischemic neuronal degeneration in vivo is limited to the delayed stage of degeneration affecting CA1 hippocampal neurons, or extends also to an acute fulminating stage of degeneration to which other neurons are susceptible. More importantly, it is an excellent model to test pathogenic mechanisms of, and prophylactic approaches to, a serious childhood condition, reversible degenerative glaucoma, cephalic palsy. Supported by Research Scientist Award RO3 EY00884 (UN) and a grant from Pfizer Pharmaceutical.


Previous studies have shown that kainate (KA) and other excitotoxins incubated with susceptible brain preparations can cause multiple neurochemical sequelae, such as decreased glutamate (GLU) synthesis and release of certain amino acids, e.g., glutamate (GLU); these in vitro effects were thought to be associated with swelling of cellular elements and could be prevented by treatment with the CI–/HCO3–-antidote IODs. The present study, central retina retinas of embryonic (E13) chick was incubated in a Krebs-Ringer-bicarbonate medium with various concentrations of KA (2-200 μM) in the presence and absence of IODs (300 or 600 μM). In the stil (A1A) retina the tissue and incubation medium were measured and correlated with histological responses. At 2 μM changes in AA were minimal; at 25 μM there was a measurable increase in medium GLU and tauone (TAU). At 50 μM KA, GLU, GLN, TAU, ARA and GABA were significantly increased in the medium. These trends were further increased at 100 and 200 μM KA. Coincubation with 300 μM IODs substantially, but only partially, prevent the release induced by 50 μM KA; 600 μM IODs offered complete protection. Histological damage was found at all concentrations of KA tested; however, just as with the AA findings there were small amounts of vacuolization both pre- and longitudinally. The radial damage was typical of KA-induced retinal lesions, i.e., mainly somal swelling in the inner and outer layers of the retina and vacuolization in the inner plerum layer (PLR). Increasing concentrations of KA produced more severe somal swelling than the INL, an increase in PLP vacuolization and involvement of other retinal populations (displaced amacrines and somas in upper regions of the INL). At 200 μM KA glial cells (GMS) swollen, as was the TNL, an increase in PLP vacuolization and involvement of other retinal populations (displaced amacrines and somas in upper regions of the INL). There was a sharply defined regional sensitivity to KA along the length of central posterior retina. There was a loss along about 50% of the tissue length. Increasing KA correspondingly increased the length of retina involved; at 200 μM KA the entire length of retina was maximally affected. A graded response was also seen in areas less sensitive to KA. Consistent with the biochemical findings, 300 μM IODs partially, and 600 μM IODs completely, blocked the KA effect on mouse retina. About 80% of the central posterior retina was maximally affected by 50 μM KA with 20% showing mild effects. Each correlate by others showed that deletion of CI– from the incubation offered similar protection. The present experiments indicate that CI– may participate in the acute excitotoxic via mechanisms more complicated than simple passive diffusion.

431.8 PHARMACOLOGICAL PROPERTIES OF A KAINIC ACID-PREFERRING RECEPTOR ON OFF-BIPOLAR CELLS IN THE CHICKEN RETINA. I.C. Morgan (SPONT: R.F. Mark). Centre for Visual Sciences and Research School of Biological Sciences, Flinders University, GPO Box 475, Canberra City, ACT 2601, Australia.

The pharmacological properties of a kainic acid-prefering receptor on bipolar cells in the chicken retina were determined by following the cell swelling and necrosis induced by intravitreal injections of excitotoxic amino acids in the retina. Acute exposure to low concentrations of kainic acid (5-10 μM) caused selective swelling of bipolar cells in the chicken retina. Long term exposure resulted in destruction of the cells, with elimination of OFF-responses at the ganglion cell level, suggesting that they are OFF-bipolar cells (Dvorak, D.R. and Morgan, I.C. Neuroscience Letters 36:249, 1983). In contrast, 100-fold higher doses of N-methyl-D-aspartic acid, quisqualic acid and RS-alpha-amino-3-hydroxy-5-methyl-4-isoxazoleacetic acid had no effect on these cells, although they were effective excitotoxic in the inner retina, where RS-alpha-amino-3-hydroxy-5-methyl-4-isoxazoleacetic acid was equipotent with kainic acid. While the lack of effect of quisqualic acid could be explained by active uptake, there is no evidence that N-methyl-D-aspartic acid and RS-alpha-amino-3-hydroxy-5-methyl-4-isoxazoleacetic acid are taken up by neural cells. Bromovalerianate, which is a powerful agonist at the kainic acid-preferring receptor on immature rat dorsal root ganglia, was without effect on the bipolar cells. The effects of kainic acid on the bipolar cells were blocked effectively by piperidine-2,3-dicarboxylic acid (PDA) and glutamate in the absence of PDA. The selectivity of the effects of kainic acid on the bipolar cells, 2-Amino-5-phosphonovaleric acid and glutamic acid diethyl ester were without effect. The selective effectiveness of kainic acid and piperidine-2,3-dicarboxylic acid at the receptors on the OFF-bipolar cells provides an interesting model for studying excitatory amino acid transmission and excitotoxicity.
431.9 NADPH DIAPHORASE HISTOCHEMISTRY IN RABBIT RETINA: EFFECTS OF KAINIC ACID. S.M. Sagar. Neurology Service, V. A. Medical Center, and University of California, San Francisco, CA 94143

We have previously shown that NADPH diaphorase (NADPHd) histochemistry in the rabbit retina selectively stains two classes of amacrine cells which can be distinguished by cell body size and intensity of staining. In the brain, NADPHd-reactive neurons have been shown to be resistant to a variety of metabolic insults, including the N-methyl-D-aspartate (NMDA) class of excitatory neurotransin. As an initial step in the evaluation of the effects of neurotoxins on retinal NADPHd neurons, kainic acid (KA) was administered by intraventricular injection to one eye of adult, male rabbits; the opposite eye received sterile saline as a control. Ten days later, the retinas were removed and hemisected vertically. Half of each retina was fixed in 4% paraformaldehyde and processed for NADPHd histochemistry; the other half was assayed for dopamine (DA) by HPLC with electrochemical detection and for protein.

At doses of 30 and 60 nmol KA, the DA concentration was reduced by 80% or more, and the large, darkly staining NADPHd-reactive cell type was substantially destroyed. However, the smaller cells were relatively spared. Because the large cells and their processes were removed, the morphology of the small cells was clarified. These cells have their dendritic arbors in the outermost sublamina of the inner plexiform layer (IPL), implying that the large cells give rise to all of the processes in the middle and sublamina of control retinas. Occasional large NADPHd-reactive cells survived. A notable feature of these cells revealed in KA injected retinas is that they have straight, sparsely branching varicose processes which extend for several mm in the IPL. At a dose of 120 nmol, many of the small cells are destroyed also.

Therefore, the small NADPHd-reactive neurons are relatively resistant to the action of KA, and histochemical NADPHd is a useful morphologic tool to explore anatomic features of these cells. The larger cells are about equally sensitive to KA as dopaminergic cells, so that resistance to excitatory neurotoxins is not a universal feature of NADPHd reactive neurons. The two NADPHd reactive cell types may receive different excitatory amino acid inputs or express different classes of receptors. Future studies employing excitatory neurotransin other than KA will explore this hypothesis further.
432.3 D-APV AND PCPINDUCED DECREASE CA1 PYRAMIDAL CELL EXCITATION PRODUCED BY NORMIDINE IN THE HIPPOCAPAL SLICE. E.S. Sweniuren, A.T. Malot, and C. Chakrav. Department of Pharmacology, University of Washington, Seattle, WA 98195.

Eletrophysiologival studies suggest that opioids increase hippocampal pyramidal cell excitation by reducing inhibitory neurotransmitter release from GABA-ergic interneurons. Opioid-induced pyramidal cell disilation also involves NMDA receptor activation as demonstrated by the ability of 0.2-amino-5-phosphonovaleic acid (D-APV), a selective NMDA receptor antagonist, to partially reduce the increase in CA1 pyramidal cell excitability produced by normidine (Sweniuren and Chakrav, Neurosci Lett.). In the present study, we have determined that D-APV has the same effect as normidine in reducing pyramidal cell excitation produced by normidine (NOR).

Extracellular recordings were made from rat hippocampus slices (500μm): CA1 population spikes were evoked by Schaffer collateral stimulation. The stimulus intensity required to produce 1/2 maximum primary response (S12) and afterpotential amplitudes were measured. NOR (10μM) added to the superfusion buffer decreased the S12/18 2.6% (mean ± SEM, n = 9) and produced a 3.19 ± 0.26 mV afterpotential (mean ± SEM, n = 11). D-APV partially inhibited NOR-induced afterpotential reduction with an IC50 of 10μM. We conclude that the NOR-induced afterpotential has an NMDA-mediated component and an additional excitatory component both of which are sensitive to PCP. This suggests that the actions of PCP, at this concentration, are not limited to NOR receptor inhibition. Interestingly, NOR-induced afterpotentials in the dentate granule cell region were completely blocked by 50μM D-APV, suggesting that the differences in the pharmacology and/or efficacy of these two regions are due to the ability of PCP to competitively inhibit NMDA receptors.

The ability of PCP to completely inhibit afterpotentials and to decrease tissue sensitivity to stimuli has been thought to be due to the production of excitatory amino acid receptors in the CA1 region. Intracellular recordings reveal that PCP (1μM) abolished spontaneously evoked spikes, reduced the membrane time constant, and increased input resistance by -0.5mA, 10mA current injection. The same concentration of PCP also abolished evoked field potentials recorded extracellularly in the absence of NOR. These effects, therefore, appear to be completely reversible.

3-PPP, in intracellular recordings, had no effect on NOR-induced depolarizations at 100μM, a concentration below the threshold to its anesthetic actions. In extracellular recordings, 3-PPP (100μM) partially blocked the NOR-induced afterpotential. The mechanism of 3-PPP action in this system is not clear but is unlikely to be NOR receptor inhibition.

This study was supported by NIH Grant NS-23483, a FMA research starter grant, and PHS NRSA S-320 07020 from NIMH.

432.4 ELECTROPHYSIOLOGICAL ANALYSIS OF HIGH AFFINITY (+/-)SKF-1047/SIGMA AND PCP/SIGMA LIGANDS ON CA1 PYRAMIDAL NEURONS IN VITRO. A.T. Malot, E.S. Sweniuren, and C. Chakrav. Department of Pharmacology, University of Washington, Seattle, WA 98195.

[3H](+/-)SKF-1047 has been shown to bind to high and low affinity sites in the hippocampus and other brain regions. The psychotomimetic properties of sigma ligands and dissociative anesthetic such as PCP are mediated through the low affinity site. Lodge and coworkers have shown that ligands acting at this site inhibit noradrenergic responses. There are also a number of studies suggesting that these ligands may have effects on potassium and possibly other ion conductances. As yet, no physiological or behavioral effects have been observed in vivo that might suggest that the CNS. We have compared the physiological and pharmacological actions of the high affinity sigma ligands, (+/-)SKF-1047 (±-3-hydroxyphenyl)N-(1-propyl)piperidine, DTG (dithyloquinine) and (+/-)SKF-1047 to PCP in area CA1 of the rat hippocampal slice.

The bath application (+/3-PPP) or (+/-)SKF-1047 at concentrations below 100μM produced no change in spontaneous activity, membrane potential, liquid resistance, spike amplitude or width. AP7 following a 1±2000 ms current pulse, threshold for orthodromically driven spikes or the amplitude or duration of the resulting IPS. 100μM DTG and PCP or 1μM (+/-)3-PPP produced a decrease in spontaneous activity, an increase in spike width, fewer spikes following depolarizing current injection and an increase in threshold for orthodromically driven spikes. 1μM DTG or PCP dramatically reduced spike amplitude and the number of spikes per current pulse and abolished the synaptically driven spike and the associated EPSP/IPSP.

Extracellular recordings of CA1 field potentials also demonstrated that bath application of (+/-)SKF-1047 DTG and (+/-)3-PPP at concentrations less than 100μM had little or no effect on either sensitivity to stimulation of Schaffer collateral or the amplitude of the orthodromically evoked primary population spike. Higher concentrations (1mM) of these compounds produced a complete inhibition of the primary spike which fully recovered within 60 min following washout.

The ability of (+/-)3-PPP, DTG and PCP to inhibit NMDA induced activation was also tested. NMDA was applied to the pyramidal cell dendrites in stratum radiatum via pressure pipette fited with 100μM drug solution. Bath applied (+/-)3-PPP (1μM) or DTG (10μM) produced no change in the amplitude or duration of the NMDA induced depolarization, while PCP (10μM) completely inhibited this response on the same cell. The NMDA response recovered slowly following washout with 10μM PCP.

Except for the PCP inhibition of NMDA responses, these compounds appear inactive at concentrations lower than or equal to 100μM. Concentrations greater than or equal to 100μM alter ion conductances, and probably represent the anesthetic properties of these compounds. The lack of effect of DTG and 3-PPP on the NMDA receptor suggests that further investigation will be required to elucidate the function of high affinity (+/-)SKF-1047 sites in the hippocampus.

Supported by NS-23483. We thank Dr. Eckard Weber for the gift of DTG.


We have previously shown that glutamate and its analogues, kainate, quinulinate, and NMDA, produce responses of varying amplitudes in isolated rat retinal ganglion cells (Alzenman et al., Soc. Neurosci. Abstr. 12:56; 1986). In order to further investigate the pharmacological properties of the postulated amino acid receptor channel complexes we studied the interactions of MK801 and NMDA in solitary ganglion cells from the rat retina by means of the whole-cell patch-clamp technique. Ganglion cells were retrogradely labelled in situ by injection of the fluorescent marker granule blue into their projection sites. The application of drugs during recording was performed by microperfusion from glass pipettes.

In the absence of extracellular Mg(2+), NMDA (200μM, the maximal non-desensitizing dose) generated inward currents (10 - 40 pA) in 68% of the cells under study when voltage-clamped at their resting potential of -60 mV. In contrast, kainate (125μM) produced larger and non-desensitizing currents (-80 to -750 pA) in all cells tested (Vh = -60 mV). When applied together, NMDA appeared to antagonize the kainate responses by approximately 50%, even in cells having no measurable response to NMDA alone.

The second finding was the NMDA channel blockers PCP (phencyclidine-dis-hydrochloride; 100μM) and MK801 (10μM) reversibly antagonized responses evoked by kainate directly. In contrast neither PCP nor MK801 influenced the responses evoked by kainate at the given concentrations. Furthermore, in the presence of NMDA and PCP, kainate generated large inward currents which were comparable in amplitude to those generated by the addition of kainate and NMDA together. Similar results were found when MK801 was substituted for PCP. Thus, kainate continued to elicit currents at a time when NMDA-activated conductances were blocked by either PCP or MK01.

Based on these findings, this system will be useful in studying the ionophores associated with excitatory amino acid receptors.

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**EXCITATORY AMINO ACIDS: PHENCYCLIDINE INTERACTION**

432.7 
**MK-801 POWERFULLY PROTECTS AGAINST N-METHYL ASPARTATE NEUROTOXICITY**

The excitatory amino acids (EAA), glutamate (Glu) and aspartate and related analogs—known collectively as excitocytotoxins—have neurotoxic activity mediated through excitatory synaptic receptors. Several EAA receptor subtypes have been identified and named according to the agonist (e.g., N-methyl-aspartate, kainate or quisqualate) with which they preferentially interact. A number of EAA antagonists have been identified, and their potencies and specificities are based on those that block the excitatory and neurotoxic activity of N-methyl-aspartate (NMA). EAA antagonists have been classified as follows: 1) mixed EAA antagonists, 2) competitive NMA antagonists, and 3) non-competitive NMA antagonists. Phencyclidine (PCP), a non-competitive NMA antagonist (Anis et al., Br J Pharm 29, 505, 1963), is the most powerful antagonist of EAA neurotoxicity previously described (O'neill et al., Neurosci Lett 89, 29, 1986). Here we report that MK-801 [(+)-5-methyl-10,11-dihydro-sib dibenz(a,d) cyclohepten-5,10-imine], which resembles PCP in electrophysiological and receptor binding properties (Hwang et al., Pharm 92, 1, 7104, 1986), is 5 times more powerful than PCP in preventing NMA neurotoxicity in the chick embryo retina. Its antagonism is specific for NMA toxicity, as it is non-competitive and does not entail inhibition of EAA receptor binding.

Endogenous excitocytotoxins have been implicated in several neuropathological conditions, including brain damage associated with ischemia, anoxia-ischemia and hypoglycemia. While more than one EAA subtype may be involved in the pathophysiology of these conditions, the NMA receptor may be of particular importance as it is the most abundant EAA receptor subtype in mammalian brain. Given the extreme potency of MK-801 in preventing NMA receptor-mediated neurotoxicity and recent evidence for its ability to prevent either seizure-mediated (Clifford et al., this meeting) or aspartic acid-mediated (Foster et al., this meeting) neuronal degeneration in vivo, it may be a valuable neuroprotective agent in clinical neuroscience. Supported by RSA MH 38884 (JW), ES 07666, DAMD 17-86-C-0010 and a grant from the Washington University/St. Louis Biomedical Res Fund. This work was generously supplied by Merck Sharp and Dohme Res. Labs.

432.9 
**THE PHENCYCLIDINE (PCP) RECEPTOR ACYLATING AGENT, METAPHTH, POTENTIATES 2-AMINO-7-PHOSPHOCHORIDATO (AP7) INDUCED STEREOTYPED BEHAVIOR AND ATAXIA IN RATS.**

The competitive N-methyl-D-aspartate (NMDA) antagonist AP7 has been shown to induce phencyclidine-like stereotyped behavior and ataxia in rats in a dose dependent and stereoselective manner. Additionally, phencyclidine and other dissociative anesthetics have been shown to be noncompetitive NMDA antagonists. In an effort to further probe the relationship between the PCP and NMDA receptors, the effects of PCP receptor acylation on AP7 behavioral responses were examined. Behavior was evaluated 24 hours after i.c.v. administration of metapt in rats. The levels of metaph that were routinely used (0.001-2.0 nmole) induced neither ataxia nor stereotyped behavior when administered alone. However, metaph, in a dose dependent manner, substantially enhanced the ability of AP7 (administered i.v. 24 hours after metaph treatment) to induce these behaviors without significantly affecting their duration. Pretreatment of the rats with 0.5 nmole of metapt lowered the ED50 for the D-AP7 induction of ataxia from 1.4 mmole/kg to 0.4 mmole/kg. NMDA receptor binding analysis on synaptic plasma membranes isolated from the forebrains of rats pretreated in vivo with metaph revealed no alteration of Bmax values when compared to control membranes. Additionally, the IC50 and Kd for the NMDA receptor was unchanged following in vitro treatment of the synaptic plasma membranes with metaph. This indicates that the metaph induced stereotyped behavior and ataxia is not due to an increase in NMDA receptor number. These results further support the functional coupling of the NMDA and PCP receptors.

432.8 
**COMPARATIVE EFFECTS OF PHENCYCLIDINE, KETAMINE AND MK-801 ON THE RAT ELECTROENCEPHALOGRAM (EEG).**

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Characteristic EEG effects from the neocortex and hippocampus were compared following phencyclidine (PCP), ketamine and MK-801. A total of 6 adult, male, Sprague-Dawley rats were chronically implanted with bipolar EEG and temporalis muscle electrodes. Each rat was placed in a plastic chamber 30 cm in diameter by 29 cm deep and recordings were obtained on polygraph paper and also recorded on tape. Additionally, EEG induced body movements and EEG data were collected simultaneously on a video/analogue cassette recorder. Cumulative doses of PCP (3.2, 10, 32 and 56 mg/kg), ketamine (10, 32, 100 and 180 mg/kg) and MK-801 (1, 3, 10 and 32 mg/kg) were administered i.p. every 15 min. Rats were used every 2-4 days to allow for drug elimination. A Latin Square design was used such that the order of drug presentation was random for each rat. Maximal doses were based on 75-80% of the estimated LD50 for each compound.

Many behavioral and EEG features common to all three compounds were observed. Small doses produced side to side head movements and stereotyped circling behavior which were accompanied on the EEG by large amplitude irregular activity from the hippocampus and large slow waves (1-3 Hz) from the neocortex. The amplitude of the electroencephalogram (EEG) was also increased. After intermediate doses of these agents, most rats were unable to support themselves. The amplitude and incidence of large, irregular activity in the hippocampus and slow waves in the cortex even increased. After larger doses, large sharp waves began to appear from both EEG leads and the animals were typically unable to right themselves. For PCP, this EEG sharp wave activity was correlated with the EEG. The largest dose of each compound produced an increase in the frequency and occurrence of all aberrant wave forms in both EEG leads. Additionally, PCP produced pronounced but brief EEG and EMG seizure activities and ketamine produced effective, general anesthesia. After 3 hr post dosing, sharp activity decreased while the incidence of background 15-20 Hz activity increased in both leads. After 7 hr gross behavior and EEG partially recovered and by 24 hr, returned to baseline levels.

It is concluded that while all three agents have similar EEG and gross behavioral features, depending on doses, there are distinct differences which make a simple classification difficult. (Supported in part by NIDR grant DA 1531.)

432.10 
**METAPHTH, AN ACTIVATING PHENCYCLIDINE ANALOG, ENHANCES N-METHYL-D-ASPARTATE STIMULATED SODIUM FLUX.**


Tetrodotoxin insensitive 22Na influx from rat hippocampal slices is stimulated by excitatory amino acid agonists such as N-methyl-D-aspartate (NMDA). The stimulation of 22Na influx from preloaded hippocampal slices by NMDA can be blocked by the subclass selective antagonist, D-2-amino-5-phosphonovalerate (D-APV) and by phencyclidine (PCP). The antagonism by PCP is non-competitive while that of D-APV is competitive (pG=4.63). PCP and D-APV do not significantly affect the 22Na influx stimulated by kainate and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid, agonists for other excitatory amino acid receptor subclasses. The PCP receptor acylating agent, metaph, given to rats in vivo (i.mole, ivh), enhanced the NMDA stimulated 22Na influx assayed 24 hours later. The basal 22Na flux was unaffected by metaph pretreatment. The non-competitive inhibition by PCP and the enhancement by metaph of the NMDA stimulated 22Na influx supports a close interrelationship of the PCP and NMDA receptors. It is possible that metaph may allosterically enhance the probability of opening of the NMDA associated ion channel or that it may have a toxic inhibition regulation by endopsychosis. The results also suggest that metaph does not bind to the PCP site as an NMDA ion channel plug as has been postulated for PCP.
Evidence has been accumulating that indicates a strong functional coupling between the phenylcyclidine (PCP) and the excitatory amino acid receptors. PCP has been shown to inhibit the neurotransmission at certain excitatory amino acids synapses (N-methyl-D-aspartate preferring) and PCP binding has been shown to be enhanced by glutamate. In attempts to gain a further understanding of these interactions we tested the effect of a known PCP receptor interacting agent, metahpit, on glutamate binding.

We report that metahpit causes irreversible inhibition of CaCl₂-dependent L-[³⁵S]glutamate binding to rat synaptic plasma membranes (SPM). Precipitation of SPM with metahpit (35µM) for 5 minutes caused a 33% decrease in specific [³⁵S]glutamate binding. The decrease in binding is time and concentration dependent. Furthermore, the irreversible nature of metahpit's inhibition is apparent as after 3 washes of the SPM the decrease in binding remains. Also, if one treats forebrain homogenates (PH2) with metahpit and then proceeds to isolate the SPM, the inhibition is still evident. This decrease appears to be selective for the 2-amino-3-phosphonobutanoate subclass of excitatory amino acid binding sites.

The results of this study provide information about the structural requirements for the anticonvulsant effects of analogs of alpha-amino-omega-phosphonocarboxylic acids.

Compounds examined included the 4,5-benzox analogs of AP6 (NDC 521), AP7 (NRC 451) and AP8 (NRC 665) as well as 5,6-benzox AP7 (NFC 517) and 6,7-benzox AP8 (NFC 657). The potency of each compound to prevent chemically- and electrically-induced seizures (male C57 mice) was determined using standard methodologies and, toxicity assessed using the rocroft procedure. All compounds were administered ICV, with testing performed 15 min after injection. Inhibition of NMDA-evoked release of ³⁵S]glutamate from slices of rat corpus striatum was studied using the method of Lehmann et al. (J. Pharm. Exp. Ther., 232, 873, 1985).

NFC 517 and NFC 657 were found to be devoid of activity in the maximal electroshock (MES) and methanol (PTZ) seizure models, whereas NFC 657 elicited convulsions when administered alone. Both NFC 451 (ED₅₀ = 430 µg/kg, MES; ED₅₀ = 91 µg/kg, PTZ) prevented PTZ- but not MES-induced seizures. In contrast, AP7 displayed anticonvulsant activity in both tests, having ED₅₀ values of 6 and 36 µg/kg in the MES and PTZ tests, respectively, whereas AP8 was effective only in the MES procedure. Like AP7, NFC 451 prevented strychnine (STN)-, picrotoxin- and bicuculline (BIC)-induced seizures with a potency only slightly less than AP7. Both AP7 and NFC 451 prevented BIC- and STN-induced mortality in convulsing animals. With the rocroft test, NFC 451 (TD₅₀ = 36 µg/kg) had a more favorable TD₅₀/ED₅₀ ratio than AP7 (TD₅₀/ED₅₀ = 1.1 µg/kg), although both compounds were toxic at convulsant doses. NFC 451 equipotent to AP7 in inhibiting NMDA-evoked ¹⁹FACH release from perfused slices of corpus striatum.

The results indicate that NFC 451 may be an NMDA receptor antagonist having a potency similar to that of AP7.

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432.12 Locomotor hyperactivity induced by frontal cortex and intraventricular injections of PCP and the NMDA antagonist, AP-7.

As the rate of kindling in the AP-7 group (25.2 ADs) was slower than in our previous groups treated with APV alone (17.3 ADs) or SCD alone (16.7 ADs), these data suggest that blocking two excitatory receptor types implicated in kindling leads to a greater retardation than blocking either type alone. This implicates a mechanism involving the summation of excitatory neurotransmission in amygdala kindling.

Supported by a grant from B.S.E.R.C. to D.P. Cain.
N-METHYL-D-ASPARTATE:

MK-801 (N-[1-5-methyl-10,11-dihydro-5,8-dibenzono[a,d]cyclohepten-5,10-anime) has been found to be highly potent in protection against maximal electroshock and bicuculline-induced seizures in mice (Climachsait, B.V. et al., Drug Dev. Res., 1983;7104-7108,1986) in a chronic seizure model. The ability of MK-801 to block seizures in amygdaloid kindled rats has been investigated in the present experiment. Male Long Evans rats (N=6) were implanted with bipolar nichrome wire electrodes in the basolateral amygdala, and stimulated (1 train biphasic square waves at 60Hz once daily until 3 to 4 generalized stage 5 seizures (receiving with bilateral forelimb clonus and loss of postural control) were observed. The following day, animals were administered 0.0, 1.0 or 10 mg/kg of MK-801 ip in physiological saline one-half hour before testing. All animals received all dosages in a counterbalanced fashion with a 72-hour interval between test days.

Behavioral responses to MK-801 prior to stimulation included enhanced motor activity and circling at the low dosage, and ataxia, paddling movements of the hindlegs and headbobbing at the high dosage. The maximal seizure stage observed in animals receiving the high dose (10 mg/kg) of MK-801 was stage 3 (unilateral forelimb clonus contralateral to the side of stimulation). Five out of six subjects demonstrated only salivation and some flexion of the digits in response to stimulation. At 1.0 mg/kg, the maximal seizure stage attained was stage 4 (receiving with bilateral forelimb clonus), most subjects displaying only stage 2 seizures. All control animals responded with fully generalized stage 5 seizures. The duration of the evoked afterdischarge recorded in response to stimulation was decreased by MK-801 from a mean of 62 sec in the control to 36 sec in the low dosage (1.0 mg/kg) groups. Increasing the dose by a factor of 10 did not produce any further reduction in duration (mean=34 sec).

These findings demonstrate that MK-801 suppresses behavioral and electrographic responses induced by electrical stimulation. In Cain (Neurosci. Abstr., 12:69,1986) reported similar effects with another NMDA-receptor antagonist. The anticonvulsant properties of MK-801 may depend on its antagonism of this receptor type.

BLOCKADE OF NMDA RECEPTORS REDUCES PSYCHOMOTOR AND POSTSYNAPTIC EXCITABILITY EXHIBITED DURING REPEATED ELECTRICAL STIMULATION OF CAI PYRAMIDAL CELLS. R.P. Poole and J.S. Kosca. Dept. of Neurology, Stanford School of Medicine and Yale Medical School, and Palo Alto VA Medical Center, Palo Alto, CA 94304. Several studies have shown that N-methyl-D-aspartate (NMDA) receptors in the hippocampus do not contribute to the post synaptic response elicited by a single stimulus in the CAI region of the hippocampus, but are activated during the high frequency stimulation used to induce long-term potentiation. We investigated the stimulation frequency-dependence of NMDA receptor activation and its contribution to changes in postsynaptic excitability during repetitive stimulation. Extracellular stimulation and recording were performed in the rat hippocampal slice preparation. Trains of stimuli (5-80 Hz, 1 sec) were delivered in the stratum radiatum of CA1, and successive responses consisting of identifiable pre- and postsynaptic components were recorded. For each stimulus train was recorded in normal Krebs' and in solution containing 100 mM DL-2-amino-5-phosphonovaleric acid (APV), a selective blocker of NMDA receptors.

In normal solution, repetitive stimulation leads to a transient frequency-dependent facilitation of the postsynaptic response (as indicated by increases in the amplitude of the population spike and EPSP), followed by a progressive abolition of the postsynaptic response. Repetitive stimulation during the facilitated phase. Blockade of NMDA receptors by APV significantly reduced activity-evoked changes in the postsynaptic response at all frequencies tested, including the occurrence of repetitive spiking. The reduction in facilitation was particularly evident at frequencies above 10 Hz. The presence of an NMDA receptor-mediated component of the postsynaptic response at frequencies below 5 Hz which contributes to facilitation of the postsynaptic response. A similar attenuation of activity-evoked changes in the postsynaptic response by APV was also observed. Since activity-evoked changes in presynaptic response have been found to correspond to changes in the extracellular potassium concentration ([K+]o), this suggests that blockade of NMDA receptors reduces increases in [K+]o stemming from postsynaptic activity. Recordings with k+ sensitive microelectrodes have thus far confirmed this conclusion.

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In several animal models of epilepsy, synaptic excitation mediated via N-methyl aspartate (NMA) receptors appears to play a key role in the genesis of epileptic activity. It has also been shown that several group I metabotropic glutamate receptors (GluR1a/2, GluR3/4) are also involved. We therefore tested the effects of MK-801, DL-2-AP5 and CGS 19755 on excitatory potentials in the neocortex of rats. MK-801 (10 mg/kg, 1.2 uM), DL-2-AP5 (50, 100, 200, 500 and 1000 uM) and CGS 19755 (50, 100, 200, 500 and 1000 uM) were given intraperitoneally. After 15 min, spontaneous epileptiform potentials were recorded in the neocortex using microelectrodes. The effects of the drugs were then compared to the baseline activity. MK-801 and DL-2-AP5 significantly reduced the number of spontaneously occurring epileptiform discharges and abolished the effect of kainate in all animals. CGS 19755 was ineffective in all animals. These results suggest that NMDA receptors play an important role in the generation of spontaneous epileptiform activity in the rat neocortex.


NMDA has been shown to evoke the release of [3H]ACh from striatal slices. The release of [3H]ACh release evoked by NMDA (10uM) was decreased by 90% at 1.2 mM MgCl2. 70% by the competitive NMDA-type receptor antagonist CGS 19755 (10 uM), 50% by 10uM MK-801, a non-competitive antagonist of NMDA receptors. The release of [3H]ACh evoked by 15 uM RCl was not blocked by CGS 19755, RMO1 or strychnine. To test the hypothesis that glycine potentiates the effects of NMDA, the concentration of glycine was increased to 10 mM. The release of [3H]ACh evoked by 100uM glycine was blocked by 1.2 mM MgCl2.

The release of [3H]ACh from striatal slices is not mediated via an NMDA-type receptor.

We have reported (JPH 130-132, 1987) that the glutamate analogues N-methyl-D-aspartate (NMDA) and kainic acid (KA) stimulate N-methyl-D-aspartate (NMDA) release from superfused rat hippocampal slices. We now report evidence that the interaction of NMDA with other transmitters in the hippocampus, we examined the effects of cholinergic drugs on spontaneous and NMDA-induced release of NMDA.

Bicuculline (50 μM), an antagonist of GABAergic transmission, had no effect on NMDA-induced release. The release of cholinergic drugs was enhanced by subtracting the release during the first three fractions from the release in the presence of drug over the next three fractions. This enhancement of NMDA-induced release was calculated by subtracting the release from the control drug containing fractions from those seen in the fractions following an NMDA pulse (100 μM, 2 min).

The nicotinic agonists dimethylphosphorylcholine (DMPC) and carbamylcholine (CCh), which are both positive allosteric modulators of GABAergic receptors, were also used to test the interactions between these transmitters. DMPC alone had no effect on NMDA-induced release of NMDA, but DMPC enhanced nicotine-induced release in a concentration-dependent manner. The enhancement of NMDA-induced release was also observed with the combination of DMPC and CCh, but the effect of DMPC was not additive with the effects of nicotine and CCh.

These results suggest that the co-administration of cholinergic drugs can modulate the effects of NMDA on hippocampal release. Further studies are needed to determine the mechanisms by which these transmitters interact to influence synaptic transmission in the hippocampus.

433.8 FACILITATION OF NMDA-INDUCED VENTRAL ROOT DEPOLARIZATIONS AND POTASSIUM RELEASE AFTER EXPOSURE TO NMDA ANTAGONISTS IN THE ISOLATED PROXIMAL CORD. J. H. Kung, J. H. Kung, and S. Davidoff. Dept. of Neurology, University of Miami School of Medicine and Neuropharmacology, VA Medical Center, Miami, FL 33101.

Excitatory amino acid receptors have been divided into three types based upon their sensitivity to specific antagonists. We have studied the effects of specific antagonists [e.g., 2-amino-5-phosphonovaleric acid (APV)] on NMDA release in the isolated proximal cord. APV is a potent antagonist of NMDA receptors and has been shown to reduce NMDA-induced release of glutamate in the ventral root. These results suggest that APV reduces NMDA-induced release by blocking NMDA receptor activation.

To further study the effects of APV, we microinjected APV into the ventral root of the rat lumbar spinal cord. APV was found to reduce NMDA-induced release in a concentration-dependent manner. The effects of APV were also observed in the dorsal root ganglia, where NMDA-induced release was reduced by 50% at 100 μM APV.

These results suggest that APV may be a useful tool for studying the role of NMDA receptors in the spinal cord and may have implications for the treatment of disorders associated with excitotoxicity.
SEROTONIN ENHANCES RESPONSES TO N-METHYL-D-ASPARTATE IN RAT NEOCORTEXAL NEURONS. J.W. Reynolds*, A. Baskys and P.L. Carlen. (SPON: G.A. Lienicka), Playfair Neuroscience Unit, Toronto Western Hospital, Adion Research Foundation, and the Departments of Physiology and Medicine, University of Toronto, Toronto, Ontario, Canada. Serotoninergic axons are distributed with a highly uniform pattern throughout the neocortex of the rat. Preliminary experiments showed that the 5-hydroxytryptophan-induced concentration-decrement increase in the extracellular field potential in neocortical slices. Therefore, we examined the possible interaction between the extracellularly applied amino acid N-methyl-D-aspartate (NMDA) in slices of the rat neocortex. (Coronal slices (400µm) of the fronto-parietal cortex of the Fischer 344 rats (4-6 months of age) were cut using a vibratome. Slices were perfused with a medium containing (mM): NaCl (124), KCl (3), K2HPO4 (1.25), MgSO4 (2), CaCl2 (2), NaHCO3 (26), dextrose (10), aerated with 95% O2-5% CO2, pH 7.4. Drugs were applied by perfusion or pressure ejection. Intra- and extracellular recordings were obtained in layers IV/V using conventional techniques. Bipolar tungsten stimulating electrodes were placed at the boundary of the cortical white matter and layer VI for afferent stimulation. In 75% of cells examined with intracellular recordings, perfusion with 10µM 5-HT did not change resting membrane potential (RMP), input resistance (Rin) or spike threshold as determined by intracellular current injection. In a few cells, 5-HT produced a 2-3 mV depolarization without an accompanying decrease in Rin. The hyperpolarization was reversible by washing. In 50% of cells tested, 5-HT produced a long-lasting increase in the EPSP amplitude. Other cells showed either no change (25%) or a decrease (25%). NMDA (100µM) was perfusion premixed at approximately 50 µM directly from the recording site, causing a depolarization with increased Rin. After perfusion with 10µM 5-HT, these cells exhibited an increased response to NMDA. The duration of a depolarizing response was doubled, and there was an increase in the frequency of action potentials generated by NMDA. This effect was long-lasting, and could not be reversed by washing the tissue for up to 60 minutes. It is suggested that 5-HT increases the sensitivity of NMDA receptors for the agonist. This effect is long-lasting, and indicates a neuromodulatory role for serotonin. (Supported by Medical Research Council of Canada and Ontario Mental Health Foundation).

343.13 ANTITISSUE AGENTS. DEXTROMETHORPHAN AND DEXTROMETHORPHAN ANTAGONIZE N-METHYL-D-ASPARTATE AT PRIMARY AMINE RECEPTORS. D.L. Hugill, D. Wang, D. Coulter*, D.W. Chol, and D.A. Prince. (SPON: K.E. Smith). Dept. of Pharmacology, Stanford Univ. Sch. of Med., Stanford, CA 94305. Antagonists to the N-methyl-D-aspartate (NMDA) receptor have anticonvulsant properties in several models of experimental epilepsy. It has been demonstrated that these effects are attributable to an interaction with NMDA, possibly via a common receptor. Among this "alpha" group of drugs is the common cough suppressant dextromethorphan (DM) and its metabolite dextrophan (DX). We therefore tested DM and DX for anticonvulsant properties in vivo, in guinea pig neocortical brain slices. Vibrated sections of sensorimotor cortex were maintained in an interface chamber at 37°C in normal oxygenated Ringer's. Prolonged ictal afterdischarges and ictal bursts were induced by perfusion with solution free of Mg2+ and recorded extracellularly. These pathological activities were blocked by both DX (1-100 µM bath; 250 µM topically) and DM (100 µM bath). The competitive NMDA antagonist, D-L-amino-phosphono-valerate (APV) also blocked by epileptiform discharges (50 µM bath; 250 µM topically). Intracellular recordings showed that these drugs block NMDA-induced depolarizations without altering intrinsic membrane properties (Coulter and Prince, Neurosci. Abstr., 1987). Intracellular multiple unit recordings showed that DX and DM blocked NMDA but not quisqualate-induced excitatory responses. Glutamate responses were partially suppressed by DX or DM. The time to onset of an epileptiform activity or NMDA responses was much longer than for APV. APV effects were washed out in minutes whereas DX or DM suppressed epileptiform activity for hours. As NMDA antagonists, these drugs represent a potentially new class of anticonvulsant for human use and may enhance or stabilize clinical effects with DM, and its effectiveness as an anticonvulsant agent, we suggest that it should be explored in a clinical trial. Supported by NIH grants NS 06477, NS 12511 and NS 01179 (CIDA).

343.14 DEXTROMETHORPHAN AND DEXTROMETHORPHAN ANTAGONIZE DEPOLARIZING RESPONSES OF NEOCORTEXAL NEURONS. ARE DX AND DM ACTING AT DIFFERENT RECEPTORS? D.L. Hugill, D. Wang, D. Coulter*, D.W. Chol, and D.A. Prince, Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305. Recent evidence suggests that blockade of excitatory amino acid receptors depresses epileptogenesis in a variety of animal models, and may also act to protect against both focal cortical and hippocampal damage. Dextromethorphan (DM), an agent found in nonprescription antihistamines, is reported to block extracellularly recorded responses to N-methyl-D-aspartate (NMDA). We therefore tested DM and DX for anticonvulsant properties in vitro, in guinea pig cingulate and sensorimotor cortex were prepared using conventional methods. Intracellular recordings from layers II-IV were obtained using 4 M K acetate-filled electrodes. Glutamate, NMDA, quisqualate, and kainic acid were applied focally either by brief pressure pulses to a broken micropipette, or by topographically controlled responses and those obtained in the presence of focally applied DM (1 µM) or DX (200 µM) were compared. All of the above excitatory amino acids depolarized cortical neurones. Kainate, quisqualate, and (for the most part) glutamate depolarizations were accompanied by increases in conductance. NMDA-induced depolarizations of small magnitude were often accompanied by apparent decreases in membrane conductance (as occasionally were small glutamate depolarizations). This conductance decrease persisted when the membrane potential was manually clamped to the resting level during NMDA application. Larger application of NMDA sometimes resulted in ' bistable ' oscillations of membrane potential between two levels, 10-20 mV apart. Puff application of DM or DX reversibly blocked NMDA-induced depolarization in some neurones but not others. These results suggest that the two agents block NMDA neurotransmission by different mechanisms, although further investigation is necessary to determine if the biological differences observed in the two agents are due to differences in the way the drugs interact with the NMDA receptor, or are due to differences in the action of the drugs on other neuronal mechanisms. These agents may be useful in the treatment of epilepsy and potential hypnotic use. Their availability and apparent non-toxicity would warrant clinical trial. Supported by NIH grants NS 06477, NS 12511, NS 07280.
It has been reported previously that L-glutamate (L-glut) excited hippocampal pyramidal cells by an action at excitatory amino acid receptors (Amrick & Bennett, 1975, Soc. Neurosci. Abstr., 10, 229, 1984). We have more directly investigated this effect by examining the action of L-pro in the isolated spinal cord of the neonatal rat.

Spinal cords were dissected from 3- to 4-day-old rats and lamellated by cutting down the ventral surface. The preparations were maintained between 25 and 26°C and superfused at 1 ml/min with medium of composition (mM): NaCl, 115; KCl, 3; d-NaHCO3, 24; CaCl2, 2.5; NaH2PO4, 1.2; D-glucose, 12, which was gassed with 95% O2:5% CO2. The 14 to 15 dorsal root was mounted on a stimulating electrode and d.c. recordings were made from the corresponding ventral root. Antagonists were applied in 2 ml pulses while antagonists were present continuously in the medium.

L-pro (1-8 mM) depolarized ventral roots of spinal cords superfused with standard medium, or in the presence of tetrodotoxin (10−6 M), in a dose-dependent manner with one-sixth the potency of L-glutamate (L-glu). L-pro was four times more potent than D-pro. Other substantial analogues of L-pro also had reduced potency. Depolarizations induced by L-pro showed no apparent desensitization. Application of L-glu prior to, during, and following prolonged superfusion of L-pro provided no evidence of a partial agonist action of L-pro. The free−free medium potentiated the depolarizing action of L-pro. L-glu and quisqualate (L-pro was antagonized by concentrations of 2-amino-5-phosphonovalerate (25 μM), Mg2+ ions (1 mM) and homocysteic acid) that depolarised N-methyl-D-aspartate−induced depolarizations. The NMDA receptor−mediated component of L-pro−induced response was estimated to be 60−70%. These data are consistent with reports of high affinity proline uptake and potassium−induced release that suggest a neurotransmitter role for L-pro. Since L-pro is a neutral compound our observations raise the possibility that acidic amino acid receptors may not require an agonist to possess two anionic groups and one cationic group.  


A recent study demonstrated a potassium−induced, calcium−dependent release of excitatory amino acid from rat brain (J. of Neurosci. 6: 2226−2234, 1986). Using intracellular recording techniques, we have reported that microiontophoretic applications of LHA induced depolarization similar to that induced by NMDA, and both depolarizations were antagonized selectively by the specific NMDA antagonist, 2-amino-5-phosphonoheptanoic acid (APH) in the caudate nucleus. Oines and his colleagues (Soc. for Neurosci. Abstr. p300, 1986) also found that LHA causes a lesion with the same cellular distribution and ionic mechanism as a NMDA lesion. On the basis of these studies, the above investigators suggested that LHA may be a natural transmitter at NMDA receptor sites. Considering similar actions of NMDA and LHA, we examined the effects of NMDA and LHA on cerebellar Purkinje cells and compared the interactions of these amino acids with various excitatory amino acid antagonists. In urethane anesthetized rats, iontophoresis application of NMDA elicited three different effects on the spontaneous activity of Purkinje neurons: an excitatory period of slow firing; an inhibition of firing; and synaptic responses consisting of excitation followed by inhibition. On the other hand, LHA elicited only excitatory period of firing of NMDA on the same neurons. When the effects of various excitatory amino acids such as NMDA, APH and LHA−mediated responses, excitatory effects of NMDA were antagonized effectively by 2-amino-5-phosphonovaleric acid (APV), ketamine, (GDEE) and APH and were not influenced significantly by 1-glutamate diethylster (GDEE). Inhibitory responses of NMDA were antagonized by APV, APH, and ketamine. LHA−mediated excitations were most by DCG and to successively lesser degrees by ketamine, GDEE, APH, and by APH. Thus, effects of LHA were attenuated less by specific NMDA antagonists. These findings indicate that major actions of LHA may not be mediated through NMDA receptor sites, least in the cerebellum. (Supported by NIH Grant RO1 NS 19296).
GLUTAMATE-LIKE IMMUNOREACTIVITY REVEALED BY MONOCOCCAL ANTIBODY AND SENSITIVE STAINING METHOD. J. Malte, 
C. V. Liu, F. Grande, M. Cunod and F. Streit (SPON: European Neuroscience Association, Swiss Brain Research Institute, University of Zurich, CH-8029 Zurich, Switzerland).

Although Glu is supposed to be a major excitatory transmitter, relatively little is known about its localization. Monoclonal Glu-like immunoreactivity (Glu-LI) at the light microscopic level by means of a monoclonal antibody (mAb) is described. - ACP: Immunoreactivity of mAb 2D7 as determined by antibody dilution tests in ELISA was more than 1000 times weaker for glutamine-, tau- and even weaker for other amino-acids and dipeptide-BSA conjugates, and hybridomas were screened by ELISA for production of antibodies recognizing Glu but not Asp-BSA. Immunoreactivity of mAb 2D7 as determined by antibody dilution tests in ELISA was more than 1000 times weaker for glutamine-, tau- and even weaker for other amino-acids and dipeptide-BSA conjugates than for Glu-BSA. Reactivity with Glu-BSA as measured in ELISA or determined by immunocytochemistry was abolished following preincubation of mAb 2D7 with Glu-BSA at micromolar concentration, whereas an about 1000 times higher concentration was needed with homocysteic acid- and even higher ones with the other amino-acid (glutamate-, tauine-, etc.) and dipeptide-BSA conjugates tested, and preincubation with free Glu was ineffective. - Semithin sections from glutaraldehyde-fixed, Plasmalemmal fixation tissue were etched and stained by a combination of the indirect unlabeled peroxidase-antiperoxidase technique and silver enhancement of the diaminobenzidine reaction product. Only this amongst several other immunohistochemical methods tolerated the brain tissue in isotonic solutions which mimicked terminal-like elements in brain regions such as hippocampus and cerebellum and which were mostly consistent with already available information on systems using Glu as neurotransmitter. Particularly striking was the staining of excised cerebellar areas to high background in fiber terminals and endings of the cerebellar granule cells.

GLUTAMATE IMMUNOREACTIVITY IN CORTICO-CORTICAL AND CORTICO-PARAFACODAL NEURONS OF RATS. E. Giuffrida* and A. Rustioni. Deps. of Anatomy and Physiology, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

Various experimental evidence supports a neurotransmitter role for glutamate in cortical neurons. This has been further demonstrated using an antiserum raised in rabbits that binds preferentially to hemocyanin with glutaraldehyde (Mepler et al., 1987). This immunoperoxidase method was used in rats and monkeys that glutamate positive cortical neurons are primarily located in layers III, V and VI (Conti et al., 1987). Since these layers contain the bulk of cortically projecting neurons, it is likely that these neurons use glutamate as neurotransmitter. It remains to be established whether or not only a fraction of projecting neurons are glutamate-positive. The position of the projection has been approached by a double-labeling technique: peroxidase immunocytochemistry to visualize glutamate, and retrograde transport of the fluorescent dye, diamidino yellow (DF) or fluorogold (FG). In rats, either DF (24 in UV) or a mixture of DF and FG (12 and 25, respectively) was injected in the cervical spinal cord, or in the dorsal column nuclei, or in the ventral nucleus of the thalamus, or in the motor cortex area 4 of WF mice. The dye uptake was studied with the aid of silver nitrate and resistant silver positive preterminals. In all cases not every cortical neuron labeled by the fluorescent dye was also glutamate-positive. The percentage of double-labeled neurons varied in different layers of the same cortical areas. In rats and monkeys, double labeled cells were mainly in layers III, V and VI of primary somatosensory cortex (51; 50% in II, 65% in III, 70% in V, in II, 65% in III, 70% in V) and in parafascicular thalamus (PAP, 65%, and in centrolateral thalamus (MOL, 35%, 65%, 62%). After chalazic injections, double-labeled cells were mainly in layers III, V and VI of primary sensory cortex (50% in II, 65% in III, 70% in V) and in centrolateral thalamus (MOL, 35%, 65%, 62%). After injection of the dorsal column nuclei, double-labeled cells were mainly in the contralateral thalamus area 4 and in the motor cortex area 4 of WF mice. The results suggest that glutamate is the main though not the only neurotransmitter in cortical projecting neurons and that the ratio of gluta to other neurotransmitter varies for different cortical layers and different projection pathways. Supported by MHR grant 16275.


A role for glutamate as neurotransmitter to at least some dorsal root ganglion (DRG) neurons has been proposed on the basis of mainly pharmacological and electrophysiological evidence. NAA-10 and NAA-16 DRG neurons are labeled with an antibody prepared against glutamates (Glu) conjugated to hemocyanin with glutaraldehyde; the larger neurons also contain substance P (DeGrouchy et al., 1987). Co-existence of Glu and SP in the same DRG perikaryon, however, does not prove that these substances co-exist and are released at the same synaptic terminal. For instance, immunoreactivity for Glu in DRG somata could be related to asthetic functions rather than neurotransmission. Experiments were therefore performed to explore more closely the co-release of these two substances by means of KN post-embedding double-labeling of terminals in the spinal dorsal horn, using antibodies against Glu and SP.

Rats were perfused with 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M phosphate buffer. Spinal cord was embeded in epoxy with or without post-fixation in 1% or 0.1% osmium tetroxide. Thin sections from samples of the superficial lamina of the dorsal horn were incubated in the first primary antisem and then in species-specific IgG adsorbed to 1 nM gold particles. After the first cycle of immunostaining, free anti-IgG binding sites were denatured by hot paraformaldehyde vapors and the grids were then incubated in the second primary antisem and in a gold probe of 20 nM. Since both antisera were raised in rabbit, after the first cycle of immunostaining, free anti-IgG binding sites were denatured by hot paraformaldehyde vapors. Both single- and double-labeled terminals were found. SP labeling is present over large granular vesicles in dense-bounded boutons contacting a single dendrite in the plane of the section, and in scalloped endings, typical of primary afferent fibers, contacting several dendrites, some of which contain vesicles. Glu-labeling is present over large clear vesicles in the axo-plasm of scalloped terminals. About 10 to 15% of the scalloped terminals are SP-labeled by both antisera. Co-demonstration of co-existence of Glu and SP gives further support to the hypothesis that the initial burst of monoaminergic excitatory potential followed by a prolonged depolarization in dorsal horn neurons is created by repetitive dorsal stimulation (Urban and Ramtie, 1984). Results from cerebellum of Glu and SP from the same DMS terminal by USPHS grant NS 12540.

434.7 ULTRASTRUCTURAL LOCALIZATION USING MONOCLONAL ANTIBODIES TO GLUTAMATE DEHYDROGENASE IN RAT BRAIN. J.E. Madin, J.R. Clements, A.J. Beitz, R.J. Westhold and A.A. Larson. Dept. of Vet. Biology, U. of Minnesota, St. Paul, MN 55108 and NIH, Bethesda, MD.

Glutamate dehydrogenase (GDH) may play a major role in the metabolism of glutamate in the vertebrate CNS. Glutamate and its analogs are neurotoxic and studies have suggested that deficiencies of GDH may be found in some individuals with oligopontocerebellar atrophy. Monoclonal antibodies to bovine liver GDH were produced and found to be immunoreactive for rat brain GDH on immunoblots and ELISA. One monoclonal antibody, GDH-2, reacted with GDH treated with either paraformaldehyde or glutaraldehyde, the fixatives that were used for tissue fixation in these studies. Immunohistochemical labeling of rat cerebellum with GDH-2 revealed intense staining of small, punctate structures identified as mitochondria following electron microscopic examination. Intensely labeled mitochondria were observed in CA3 and CA1 pyramidal cell glia in the cerebellum. Rarely, labeling of mitochondria in neuronal profiles, predominantly dendrites, was observed in the cerebellar cortex. Large numbers of unlabeled mitochondria were observed in many cell types throughout the cerebellum. The densely labeled mitochondria were observed in neurons and glia of other regions of the CNS including the hippocampus. Immunocytochemical staining was inhibited by preincubation of the antibody with GDH. The intense immunolabeling of a subpopulation of mitochondria found in only a fraction of the glia and neurons present in the CNS suggests that GDH may be present in high concentration in this subset of mitochondria. The presence of GDH in a subset of mitochondria may prove to be important in understanding the pathophysiological mechanisms associated with a variety of excitotoxins-related disease processes. Supported by USPHS grants NS01105, NS07813, DA04090, DE00663 & NS19208.


Studies on the excitatory amino acid glutamate have suggested that this transmitter is inactivated and recycled back into glia where it is converted to glutamine. The use of antibodies directed against amino acids has made it possible to study aspects of the "glutamatergic" system using immunohistochemical methods. We have extended the use of these antibodies to include the detailed physiological characterization of glutamate release by neurons and the subsequent uptake by glia. Slices of rat or guinea pig hippocampi (400) were incubated in Kreb's solution and continuously bubbled with 95% O2:5% CO2. The slices were then placed in an experimental incubation medium at 30°C for 1 hour; after which the slices were rinsed, fixed with aldehydes and re-sectioned at 40 μm. The sections were then treated using an affinity-purified antiserum directed against glutamate-IR. In sections incubated in control for 60 min, dense glia-like immunoreactivity (Glu-Li) was seen in the stratum radiatum, oriens and the inner molecular layer of the dentate gyrus. Intermediate staining was seen in the outer molecular layer and stratum lucidum while very little staining was observed in the middle molecular layer and the stratum lacunosum-moleculare. In sections incubated in control for 45 min at 55 mM [K+] was a loss of laminar Glu-Li and an appearance of intensity and specificity of specific glial-like staining. Labelling with a monoclonal GPAP antibody and glutamine antiserum confirmed the glial identity of these cells that do not appear to occur in the absence of Ca2+.

In slices that were exposed to high [K+] for 60 minutes and then placed into control buffer with the addition of glutamine (0.5 mM) for an additional 30 min, the "normal" pattern of Glu-Li staining was re-established. The [K+] induced appearance of Glu-Li in astrocytes was blocked by 1 mM D-aspartate, D.J. thio-β-hydroxyaspartate, cysteic acid and SITS. The staining of the glia was not affected by D,L-APB (200 μM). The addition of exogenous glutamine (50-500 μM) to control buffer induced astrocyte staining only in the absence of TTX (0.5 μM), indicating that this staining was a result of glutamate stimulation of glialneuronal. Similar results were seen with the addition of 100 mM NMDA. Depolarization of the slices by veratridine (100 μM) resulted in the depletion of the normal Glu-Li staining pattern, however, no astrocyte staining was observed, indicating that Na+ channels are required for uptake or retention of Glu-Li by astrocytes. Using this novel approach to study glutamate uptake, we have provided evidence to support the idea that transmitter pools of glutamate are "cycled" through astrocytes and that the transport of glutamate is regulated by a specific uptake site(s) which is amenable to pharmacological intervention. Supported by grants from the NIA (AG-00996) and NIMH (16961).
Excitatory Amino Acids: Localization and Release

434.9 EFFECTS OF Kainate and N-Methyl-D-Aspartate on the Release of Trictium from Rat Retina Fragments Prelabelled with 3H-Choline. L. L. TRUE AND M. R. FOREMAN. Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN, 46285.

Glutamate and aspartate have been proposed to be transmitters of bipolar cells within the vertebrate retina (Ikeda and Sheardown, Vision Res. 25:1161-1174, 1983). These transmitter candidates have been localized to the inner segments of amacrine cells by the use of receptor ligands and radioimmunoassays. The finding of NMDA receptors on these cells suggests that these transmitters may also act as autoregulators.


NAAG is an acidic peptide that has been proposed to play a role in excitatory synaptic transmission within the rodent CNS. Regional variations in NAAG levels across the rat CNS have been documented by HPLC techniques. Sensitivity constraints prevented the determination of NAAG in quantities below the nmol level. Accordingly, we have developed a liquid phase radioimmunoassay (RIA) capable of detecting pmol quantities of NAAG.

Methanol extracts of rat tissue samples were lyophilized, and NAAG was quantified by HPLC. K. C. Mallory et al., J. Neurochem. 2000; 74:970-976. We used liquid phase radioimmunoassay. The RIA was performed with NAAG as the standard and [3H]-NAAG (52mCi/mmol) as the displaceable radioligand. Tissue extracts, in amounts yielding 40-60% displacement, were incubated with ammonium sulfate precipitated immune serum (BBRL09) and [3H]-NAAG (total volume of 200 μl). After 16 h incubation, complexes were separated by filtration with a goat-anti-rabbit gamma globulin, followed by filtration.

NAAG displacement followed a sigmoidal curve with an IC50 of 4.5-6.5 pmol per tube. The sensitivity of this assay was 1 pmol per tube. N-D cyl-aspartyl glutamic acid (NAA) and aspartyl-glutamate displacement curves paralleled the curve obtained for NAAG with IC50's of 350 pmol and 75 nmol, respectively. Glutamate and aspartate displaced less than 10% at 500 nmol. Thus, this assay was highly specific for NAAG with respect to two major brain transmitters, glutamate and aspartate, and showed 50-fold specificity for NAAG with respect to NAA.

Comparison of regional NAAG levels determined by HPLC and by RIA revealed a high correlation (r = 0.99). Levels of NAAG-like immunoreactivity determined by RIA were approximately 3 nmol/mg protein higher than those measured by HPLC. RIA analysis of HPLC fractions from several regions revealed only two peaks of immunoreactivity, co-chromatographing with NAAG and NAAG-C. Correction of RIA displacement values by subtraction of endogenous NAAG crossreactivity yielded for four regions a close agreement between RIA and HPLC and by RIA across eight brain regions (r = 0.98, slope = 1).

We have performed functional neuronal tissue from various brain nucleii including the Raphe and red nuclei, the locus ceruleus, the dorsal and ventral horns of the spinal cord, striatum, and cortex. These data show a correlation between NAAG concentrations and ECS and NMDA peak concentrations in various brain regions. The relationship between NAAG-like immunoreactivity and ECS and NMDA peak concentrations appears to be linear.

434.11 EXCITATORY AMINO ACID STIMULATED RELEASE OF [3H]-GAMMA-AMINO-ButyRIC ACID FROM HIPPOCAMPAL NEURONS IN VITRO. K. M. Harris* and R. J. Miller (SPON: R. McGeer) Department of Pharmacology and Physiological Sciences, University of Chicago, Chicago, IL 60637.

Various amino acids have been suggested to be excitatory neurotransmitters in the mammalian CNS. Calcium ions are known to be a major mechanism of action of these amino acid neurotransmitters has been extensively studied in the hippocampus. One population of interneurons in the hippocampus utilizes glutamate as their neurotransmitter (GABA) as a neuropeptide. In the present study, we have investigated the release of GABA from hippocampal neurons and its regulation by extracellular glutamate. For (embryonic day 18) rat hippocampus were dissociated, grown in primary culture and used experimentally after 8-10 days in vitro. Immunohistochemical studies showed that hippocampal neurons are present in significant numbers in these cultures. Cultures of hippocampal neurons accumulated and stored [3H]-GABA and [3H]-glutamate. Preaccumulatation [3H]-GABA was released when the cells were depolarized with either 50 mM K+ or 30 μM veratridine, however [3H]-glutamate release was not detected. [3H]-GABA release was blocked by 100 μM pCPA. The dihydrokynurenone, BA 5644 and nifedipine, 1 μM had no effect on 50 mM K+ stimulated release. 3 μM tetrodotoxin (TTX) blocked release stimulated by 30 μM veratridine but had no effect on 50 mM K+ stimulated release. Veratridine stimulated release was significantly greater than release stimulated by 50 mM K+ (p < 0.05). 50 mM glutamate released significant amount of [3H]-GABA release was also stimulated by 100 μM glutamate and by 100 μM 3-Methyl-D-aspartate (NMDA). Glutamate stimulated release was slightly greater than release stimulated by 50 mM K+ (p < 0.05). Neither 100 μM or 10 μM nifedipine (3 μM or 0.1 μM) had any effect on the excitatory amino acid stimulated release. NMDA stimulated release could be blocked by 1 μM Mg2+ and a competitive NMDA receptor antagonist, 2-amino-5-phosphonopropionate (APP, 10 μM). Changes in [Ca2+] were monitored in single hippocampal neurons with the fura-2 based microspectrofluorometric technique. Basal [Ca2+] in these cells are generally above 100 nM. [Ca2+] increased to 234 mM and 291 mM when the cells were stimulated with 50 mM K+ and 30 μM veratridine, respectively. The veratridine response was blocked by 3 μM TTX. NAAG and glutamate caused dose dependent increases in [Ca2+], E[Ca2+] = 3 μM and EC50 = 10 μM, respectively. Maximal effective concentrations of NMDA (100 μM) and glutamate (100 μM) raised [Ca2+] to 241 mM and 315 mM, respectively. The response to NMDA (10 μM) was also enhanced by 30 μM glutamate. Thus, GABA can be released from cultured hippocampal neurons by raising [K+] and by excitatory amino acids. Ca2+ influx via ionotropic glutamate receptor channels appears to play a role in both responses.
CHARACTERIZATION OF POTENTIAL NEUROACTIVE SUBSTANCES RELEASED FROM RAT BRAIN IN VITRO, W. D. Koller*, R. O. Doel, H. Goldinger*, R. E. Winterhalter* and M. E. Gaudin, Brain Research Institute, Univ. Zurich, CH-8032 Zurich, Switzerland.

As no established neurotransmitters have been assigned to some major CNS pathways, a screening of neuroactive substances has been performed by selecting compounds which exist in brain slices in an ATP-dependent manner (Do, R.O., Hattenberger, M., Steilt, P. and Gaudin, M., J. Neurochem. 46: 779, 1986). 9-Fluorenylmethoxycarbonyl (FMOC) pre-column derivatization was chosen to analyse the presence of all secondary amines with reversed-phase HPLC. (4) FMOC-derivatives can be protected under mild basic conditions, thus the original compound can be recovered in high yield and can be used for further analytical purpose. Compounds which showed a Ca dependency were collected from successive HPLC runs, purified at different pH of the mobile phase and structurally elucidated by amino acid analysis and fast atom bombardment mass spectrometry. One of these compounds was identified as 9-Fluorenylmethoxycarbonyl FMOC-glutamyl glutamate, a t-dipeptide that was produced side-product of FMOC-derivatization of glutamate. Furthermore a group of substances could be isolated which are active in the presence of the protease inhibitor lactoamidase. P25c was shown to contain at least Glx and a base label group. P25c gave after hydrolysis with 6N HCl an unusual polar amino acid as a constituent.

EXCITATORY AMINO ACIDS: LOCALIZATION AND RELEASE

434.14 ELECTROACOUPUNCTURE EVOKE RELEASE OF GLUTAMATE IN THE HIPPOCAMPUS MONITORED WITH DIASTIZATION-PERFUSION IN VIVRO. D. Z. Lee*, J. M. Lazurevicz* and A. L. Chuan. New York University School of Medicine, New York, New York. Bionex., Dept. of University of Missouri, Columbia, MO 65203 and Medical Research Center, Polish Academy of Science, Warsaw, Poland.

Electroacupuncture (EA) has been used for the amelioration of pain. The EA effect is not similar to analgesia produced by electrostimulation. We have demonstrated previously that EA stimulation at acupuncture point ST-36 alters catecholamines in different brain regions and does not release Glu from hippocampus. In this study, we have demonstrated that EA stimulation mediates enhancement of Glu release in hippocampus in vivo. We have employed a new analyze technique to study the Glu release (9). We have demonstrated that EA stimulation mediates enhancement of Glu release in hippocampus in vivo. We have demonstrated that EA stimulation mediates enhancement of Glu release in hippocampus in vivo.

RAT POTENTIAL COMPOUNDS

434.13 CHARACTERIZATION OF POTENTIAL NEUROACTIVE SUBSTANCES RELEASED FROM RAT BRAIN IN VITRO. W. D. Koller*, R. O. Doel, H. Goldinger*, R. E. Winterhalter* and M. E. Gaudin, Brain Research Institute, Univ. Zurich, CH-8032 Zurich, Switzerland.

As no established neurotransmitters have been assigned to some major CNS pathways, a screening of neuroactive substances has been performed by selecting compounds which exist in brain slices in an ATP-dependent manner (Do, R.O., Hattenberger, M., Steilt, P. and Gaudin, M., J. Neurochem. 46: 779, 1986). 9-Fluorenylmethoxycarbonyl (FMOC) pre-column derivatization was chosen to analyse the presence of all secondary amines with reversed-phase HPLC. (4) FMOC-derivatives can be protected under mild basic conditions, thus the original compound can be recovered in high yield and can be used for further analytical purpose. Compounds which showed a Ca dependency were collected from successive HPLC runs, purified at different pH of the mobile phase and structurally elucidated by amino acid analysis and fast atom bombardment mass spectrometry. One of these compounds was identified as 9-Fluorenylmethoxycarbonyl FMOC-glutamyl glutamate, a t-dipeptide that was produced side-product of FMOC-derivatization of glutamate. Furthermore a group of substances could be isolated which are active in the presence of the protease inhibitor lactoamidase. P25c was shown to contain at least Glx and a base label group. P25c gave after hydrolysis with 6N HCl an unusual polar amino acid as a constituent.

434.15 A SIMPLE AND RAPID METHOD FOR MEASURING ASPARAGINE AMINOTRANSFERASE ACTIVITY IN SPECIFIC REGIONS IN THE NERVOUS SYSTEM. D. Garrison*, J. Beattis* and M.A.A. Wambourdi, Dept. of Biology, Georgetown University, Washington, D.C. 20007.

Asparagine aminotransferase (EC 2.6.1.1., NACAT) catalyzes the transamination of aspartate in the presence of keto glutarate to form oxaloacetate and glutamate. Based on immunohistochemical localization data, it has been proposed that NACAT is a marker enzyme for aspartate/glutamate transmitter pathways in the nervous system. However, only limited progress has been made in understanding the possibility of NACAT in the regulation of the synaptogenesis of the neurotransmitter pool of aspartate/glutamate. This is due, in part, to the lack of a simple and sensitive method for measuring changes in the activity of NACAT in specific areas of the nervous system under different physiological and pharmacological conditions. We report here an assay procedure which has been developed recently in our laboratory.

To assay NACAT activity, a 10 ul aliquot of potassium phosphate buffer (0.1 M, pH 7.4) containing L-[2-14C]-aspartate (50 µCi/mole, 20 µM) and -keto glutarate (20 µM) is incubated (30 min. 37 C) with a 10 ul aliquot of rat brain homogenate prepared in the phosphate buffer containing NP-40 (0.1%). Following incubation, the reaction is terminated by adding HCI (0.1N, 100 µl). As a result of the transamination of aspartate, which results in the formation of oxaloacetate, the tritium atoms at positions 2 and 3 of aspartate are released into the aqueous medium. The released tritium is separated from the radiolabeled aspartate by passing the reaction mixture over a cation exchange column (BioRad AG 50 W-X8, 7 x 30 mm) equilibrated with HCl (0.05M). The column is washed with 2 HCl and the aspartate activity in the combined flow through and wash is determined. Using unlabelled HCl followed by evaporation, we determined that this radioactivity is associated with water. Also, we have found that when 14C-aspartate, instead of 2,3-14C-aspartate, is used in the assay, the radioactivity in the product migrates with oxaloacetate in anion exchange HPLC. The product formation, as determined by the amount of tritiated water formed, was found to be linear with time up to 120 min and with tissue concentration in the 0.05 to 100 µg range. Using an extensively dialyzed brain homogenate preparation, we have found that product formation is dependent on the concentrations of both aspartate and -keto glutarate. Using this assay procedure it should be possible to accurately measure NACAT activity in specific cell zones and projection pathways in the nervous system. Thus the development of this simple and sensitive assay procedure is expected to stimulate study the role of NACAT in the syntheses of aspartate/glutamate in putative acidicergic neuronal pathways. Research supported by NIH Grant DA 02297 to J. H. Neals.
435.1 ALTERED RESPONSIVENESS OF CAUDAL NEURONS TO IONTOPHORETICALLY APPLIED NEUROTRANSMITTERS IN AGED CATS. M.E. Levine, C. Cepeda, C.D. Hall, and H.A. Buchwald. Mental Retardation Research Center, University of California, Los Angeles, 760 Westwood Plaza, Los Angeles, CA 90024.

Previous studies from our laboratory have demonstrated that decreases in excitability occur in caudate nucleus neurons in aged cats. These changes may be mediated by post synaptic and/or presynaptic alterations. In order to provide additional information, we have employed the technique of extracellular application of neurochemicals to neurons located in the rat striatum. Our results indicate that decreases in excitability occur in caudate neurons in aged cats. These decreases may be related to post synaptic alterations.

435.2 DECREASED EXCITATION IN STRIATAL NEURONS IN AGED RATS REVEALED BY IONTOPHORETIC RECORDING. C. Cepeda*, J.P. Walsh, N.A. Buchwald, C.D. Hall, R. Fish, and M.E. Levine (SPON: P.W. Hallenberger). Mental Retardation Research Center, UCLA School of Medicine, 760 Westwood Plaza, Los Angeles, CA 90024.

Previous electrophysiological studies from our laboratory have demonstrated reduced excitation in caudate neurons in aged cats. These extracellular recordings were characterized by post-synaptic reductions in the density of excitatory responses. In the present experiment intracellular recordings were used to characterize the changes in membrane potential responses to iontophoretically applied excitatory amino acids. Several changes were noted.


A mutant strain of rats that carries an autosomal recessive genetic defect is characterized by a progressively developing tremor, ataxia and increased muscle tone in the hindlimbs (Niswender et al., 1976). A disturbance in the glial amino acid transport (see Hiraike et al., 1985). This study provides a set of quantifiable indices of the impairment of motor function of the progressions of these animals and a baseline from which the results of treatments designed to ameliorate the disorder can be determined. To date we have quantified behavioral changes in 9 affected rats and 1 normal littermate (homozygote controls). None of the behavioral responses described below are displayed by non-affected littermates. The first detectable sign of the disorder is a decrease in weight gain of affected rats relative to their littermates of the same sex. Then begins at the 4th postnatal week. During the 4th and 5th week motor symptoms appear. The first symptom is a tremor of the forepaws which occurs for the duration of the rats' survival. Jaw tremor begins at about 10 days and lasts for the duration of survival. These behavioral symptoms involve increased spontaneous activity within the loss of forelimb support begins at 35-40 days and maximizes at about 60 days. The forelimbs become clenched at 45 days and remain in that position for the duration of the disorder. At 35-40 days the cats lose hindlimb support forward and under their body, display ataxia and locomote by hopping. These behaviors disappear after 60 days as limb support is increased again. The loss of limb support occurs at 45-50 days and is complete by 65 days. Hindlimb rigidity occurs at about 60 days. At 45 days of these types of severe as the disorder progresses, Animals have difficulty eating and drinking by 60-70 days and must be handled. Animals were sacrificed at about 90 days. These suggest that the genetic disorder progresses in an orderly fashion, first affecting forelimb and jaw musculature, then forelimb and hindlimb support, locomotion and finally producing rigidity of the hindlimbs. Preliminary observations of dopaminergic and immunohistochemical staining for glutamic acid decarboxylase, the synthetic enzyme for GABA, revealed decreased terminal staining in the substantia nigra. This genetic defect has the potential to serve as a model for an inherited disorder of the basal ganglia such as Huntington's disease. Supported by UHRF Grant HD05998.

The initial observation and subsequent fetal development of substance P (SP) within the basal ganglia (BG) of cats occurs before the development of other neurotransmitters (GABA, ACh, and glutamate). These events precede the formation of synapses in the BG. Thus, the role of SP during development, we analyzed its regional disposition and development in the output nuclei of the (BG) globus pallidus (GP), entopontine nucleus (SN) and substantia nigra (SN). Light and electron microscopic analysis of substance P-like immunoreactivity was performed in 16 fetal kittens of ages P5 through P80. The fetal period was defined as 0-65 days. The most important sources of SP within the BG are the neostriatal (NE) medium spiny neurons that provide the principal inputs to the mature GP, SN and SN. GP, SN and SN contained sparse SP immunoreactivity at P10 in the form of SP fibers and growth cones. These increased in frequency with fetal aging. Within the GP, SP developed along a caudal-to-rostral and medial-to-lateral gradient. The SP fibers developed in the SN from a central core region which extended and became more dense with age. At P30, the SN contained dense SP-positive fibers and growth cones emanated from the cerebral peduncle. A dense neurite meshwork of fibers, punctae and growth cones developed rapidly thereafter in all parts of the SN. Interestingly, SP-immunoreactive cell bodies were seen within the fetal SN and GP throughout development, but they disappeared with advancing age. Such cells have not been observed in the SN or GP of adult cats. They may express SP transiently during fetal development, or the expression of SP may be masked by dense SP innervation.

These data show that substance P is expressed in the target nuclei of the GP at about the same age in which NaP axons approach these targets. The early expression and development of this peptide suggests that substance P may serve as a trophic factor during the formation of the GP. Synaptogenesis may permit its subsequent function as a neurotransmitter/neuromodulator in adults. Supported by USPHS R01 NS07696, R02 NS595 and AR01358.

435.6 FINE STRUCTURE OF THE OPOSSUM SUBSTANIA NIGRA PARS LATERALIS, T.P. Ma and J.C. Barlett, Department of Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit, MI 48201.

The substantia nigra pars lateralis (SNL) of adult opossums (Didelphis virginiana) was examined with the electron microscope. Small cells (~9 μm long axis) had round nuclei with clumps of heterochromatin and prominent nucleoli. A thin rim of cytoplasm contained all commonly observed neuronal organelles except Rial bodies. Medium to large cells possessed round to oval nuclei with prominent nucleoli. A few of these nuclei exhibited indented nuclei. All cells contained large, dense, round mitochondria, often prominent, including prominent Nissl bodies. These were particularly prominent at juxtaplomerular positions opposite the emergence of dendrites, were located beneath the somata, and were difficult to contact the somata; however, dendrites were usually embedded by endings. Five types of presynaptic profiles were observed. Types A and B were asymmetrical while Types C, D, and E were symmetrical. Type A terminals contained oval to round clear vesicles (40-60 nm), a few large dense core vesicles, and mitochondria. Type B endings possessed oval to round clear vesicles (20-40 nm) but lacked dense core structures. Both Type A and B terminals contacted dendrites of all sizes which always exhibited prominent post-synaptic thickenings with subjunctival bodies. Type C endings had equal numbers of clear and dense core vesicles. The presynaptic profile contained round to oval clear vesicles (40-60 nm), dense core vesicles, and mitochondria and contacted dendrites and axons. Type D endings were large, contain many mitochondria and only contacted proximal dendrites and cell bodies. The vesicles were small and pleomorphic and accumulated adjacent to numerous short active zones. Type E synaptic profiles possessed a "core" region which had a crystalline-like array of vesicles (35-50 nm) and a "peripheral" region which contained round to oval vesicles (40-60 nm), large dense core vesicles and mitochondria. These endings which contacted dendrites and somata exhibited multiple presynaptic thickenings. Injections of HRP into the superior colliculus labeled medium to large sized neurons, and large and intermediate sized peripheral endings had five types of presynaptic profiles contacted the labeled neuronal elements. These results are similar to those described for the substantia nigra pars reticulata (SNR) in other species. The principle differences are (1) the morphology of the nuclear envelope and (2) the presence of a "core" region in the SNr profiles. This lends support to the hypothesis that the SNr may serve similar functions in the modulation of basal ganglia output.

(Supported in part by a grant from the Michigan Eye-Bank and Transplantation Center.)

435.7 LARGE PHA-L-LABELED PALLIDAL TERMINALS FOR SYMMETRICAL SYNAPSES ON THE SOMATA AND DENDBRITES OF SUBTHALAMIC AND SUBSTANTIA NIGRAL NEURONS, H. King and S.T. Kirda, Departments of Anatomy and Neurosurgery, The University of Tennessee, Memphis, College of Medicine, Memphis, TN 38163.

Projections from the globus pallidus (GP) to the subthalamic nucleus (STH) and the substantia nigra (SN) in rats were studied using Phascolus vulgaris Leucoagglutinin (PHA-L) anterograde tracing method. PHA-L was immunoperoxidase demonstrated in the GP of anesthetized rats. After two weeks of survival, rats were perfused transectially with acetate-borate buffered solutions containing 4% paraformaldehyde and 0.15% glutaraldehyde. Parasagittal or horizontal sections (40 μm) were cut using a microtome. The sections were processed for immunohistochemical demonstration for PHA-L using a peroxidase-labelled goat IgG (1:200) in PBS. All sections were processed for dual labeling of PHA-L and tyrosine hydroxylase (TH). In this case, PHA-L was first visualized using peroxidase-antiperoxidase method with DAB (brown reaction) and then TH was localized by avidin-biotin-peroxidase method with cobalt-DAB (blue reaction).

Light microscopic observation revealed that the PHA-L labeled GP axon plexus in the STH and SN consisted of thin fibers with boutons en passant and terminals. Most terminals were large (over 1 μm). In SN, PHA-L labeled terminals surrounded not only TH immunoreactive neurons but also non-reactive neurons.

Electron microscopic analysis revealed that the morphological features of the labeled processes were very similar in the STH and SN. Labeled fibers were either myelinated or unmyelinated. Labeled terminals were large and contained well preserved mitochondria, and suggested that the function of this pathway may be inhibitory. If so, the density of this projection, and its term of distribution on the pyramidal cells of the cells indicates that this may play an important role in the firing of SN neurons.

Supported by NIH grants NS20702 and NS23886.


Direct projections from the substantia innominata (SI) to dorsal mesencephalic tegmentum, specifically the pedunculopontine tegmental nucleus (PPT), have been described recently, but the detailed organization of connectivity of this pathway has not been fully documented. Moreover, the relationship of tegmental SI neurons with cholinergic SI neurons that invade this forebrain area is not understood. The experiments were designed to investigate patterns of connectivity between neurons in the sublenticular substantia innominata (LSI) and ventral pallidum (VP) and neurons in the PPT. First, the anterogradely transported lecinthin Phascolus vulgaris leucoagglutinin (PHA-L) was incorporated into VP. In light of this, the labeled terminals were followed either a ventral course through the caudal ventral tegmental area and rostral tuberal nuclei or a dorsal course through ventromedial central gray; these fiber pathways exited at the level of the decussation of the superior cerebellar peduncle (SCP) and terminated massively in the PPT extending into the lateral tegmental region. Second, implants of fluorescent tracer fluoro Gold (FG) were placed in the PPT at the level of the decussation of the SCP. Retrogradely labeled neurons were obtained in the ventral and dorsomedial pallidum, SLSI, and nucleus interstitialis of the ant. lenticulata. Co-antitropographic axonal tracing and Texas Red-cholinoic acid ethylsterase (CHAT) immunofluorescence revealed that these tegmental neurons are noncholinergic. This was confirmed by making connections with compartments of the basal ganglia. The hypothesis that PPN-SI axon terminals were cholinergic could not be supported by the data. In other studies, the total number of labeled neurons were cholinergic and were located in pars compactus, closely associated with the ventrolateral border of the decussation of the SCP. Retrogradely labeled neurons were present in the PPN (pars dissipata and pars compactus). Texas Red-CHAT immunofluorescence exhibited that ca. 20% of the total number of labeled neurons were cholinergic and were located in pars compactus, closely associated with the ventrolateral border of the decussation of the SCP. Retrogradely labeled neurons were also present in the lateral dorsal tegmental nucleus (LDTg), but <5% of these neurons were seen in the LDTg. These findings indicate that the PPN has a reciprocal connection with the SI and VP, parts of the basal forebrain related to parasitism and loops. Moreover, cholinergic neurons of the SI and PPN do not appear to make substantial contributions to these circuits.
435.9 ULIHERTRONICAIE OF THE NUCLEUS TENSORrrnut PEUDOCOLOPOLICUS IN THE RAT. B.M. Spann and J. Czepofo. Dept. of Anatomy, Michigan State University, E. Lansing, MI.

Numerous light microscopic studies have focused on the interrelationships between the nucleus tensorium pseudoculoponticus (FPN) and the basal ganglia (BG) and several hypotheses on the functional significance of these connections have been postulated. To date, there has been little electrophysiological or anatomical confirmation of the termination of the FG afferents within the FPN nor any normal baseline data on the ultrastructural organization of this nucleus. The present study reveals normal ultrastructural features of the rodent FPN and presents experimental data on the morphology and termination of the FPN afferents from the substantia nigra.

The ultrastructural analysis was performed in well-defined samples selected from sagittal sections through the lateral, medial, and ventral portions of the FPN. The observations suggest that the FPN afferents terminate almost exclusively on the dendrites since the synaptic terminals on the cell bodies are extremely rare. The FPN afferents are composed of an abundance of myelinated fibers of varying diameters, small bundles of unmyelinated fibers and a lattice of dendrites which are rather sparsely covered by nerve terminals. Three morphologically distinct types of terminal boutons have been observed so far. The most common are small (<1 m) Type I terminals which contain densely packed round synaptic vesicles and establish asymmetrical synapses with both thick and thin dendrites. The Type II boutons are larger and less frequent than Type I. They contain round synaptic vesicles which tend to be arranged in clusters and are involved in asymmetrical synapses which are characterized by the presence of a regular array of presynaptic densities and postjunctional dense bodies beneath the post-synaptic density. In addition to the synaptic junction, they often exhibit a more puncta adherens. Type III terminals consist of clusters of small vesiculated profiles and several mitochondria in a clear matrix. They form symmetrical junctions with perisomatic and dendritic terminals. In Type III, there is a case. In Type III, the Type I terminal was seen synapsing on the cell body in the median region of the FPN. However, the only afferent terminals are present throughout the nucleus, the Type III terminals are more frequently encountered in the samples from the intermedial and ventral portions of the nucleus.

The origin of the Type I and Type II terminals is at present unknown. Concerning the Type I terminals, our experimental evidence indicates that they are at least partially of nigral origin since boutons of similar morphology were labeled following iontophoretic injections of Phaseolus vulgaris leucoagglutinin (PHA-L) into the substantia nigra pars reticulata. Supported by N.I.H. Grant NS19483.


Several lines of evidence have indicated that the pars reticulata of the substantia nigra (SNR), which represents one of the output nuclei of the basal ganglia, is involved in the control of various aspects of the motor system. The SNR has a prominent control over movement systems, which is mediated through the nigro-thalamocortical, nigroreticulotegmental, and nigro-remotorpathways. Major goal of the present study was to accurately define the nigral efferent projections of the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L) which provides labeling of single fibers, terminal pleuroms and variabilites.

Single or multiple iontophoretic injections of PHA-L were made into the SNR through stereotactic coordinates of Paxinos and Watson (1986). Following survival periods of 10-14 days, the animals were perfused intracardially by a fixative consisting of 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1M phosphate buffer. The brains were cut in 30 μm thick sagittal sections on a vibratome and reacted immunocytochemically using a modified protocol by Gerfen and Sawchenko (Brain Res. 290:219-238, 1984).

Examination of the light microscopic material confirmed previous reports of SNR projections to the ventromedial and intralamellar thalamic nuclei, superior colliculus, central gray, mesencephalic pedunculoponticus (FPN) and midbrain reticular formation, and in addition, revealed several hitherto unknown targets of nigral efferent fibers. In the thalamus, a particularly dense terminal plexus was seen in a crescent-shaped area in the rostral portion of the nucleus ventralis lateralis. Furthermore, dense patches of labeled terminal fibers were consistently present in the dorso-ventral portion of zona incerta including the paracaeruleus which is known to project to the spinal cord. In the midbrain, fine labeled fibers were seen in the paracentral area in the dopaminergic cells and smaller neurons in the red nucleus. Terminal plexus was also noticed in the midline nuclei. In the striatum, the three prominent areas that were covered by the nigrostriatal fibers were in the rostral interstitial nucleus of the pons, most of the nigral fibers terminated in the FPN and only a modest number extended to the lateral and ventral portions of the nucleus.

Cortical and subcortical afferents to the caudate nucleus of 6 cats were studied by labeling the locations of retrogradely labeled cells after injection of a tiny pellet of WGA-HRP embedded in polyacrylamide gel into different regions of this nucleus. The labeled cells and their projections were traced using the standard TRITC protocol after a 48 h survival time.

Injection into either the dorsolateral (DL) or ventromedial (VM) head of the caudate from A17 to A15 (B0-5) or the dorsal posterior (DP) portion of the caudate at A15.3 (B1-1) resulted in labeled neurons within the thalamus and substantia nigra. The following corticostriatal afferents were labeled by retrograde injection of WGA-HRP into the caudate nuclei previously described by Wilson and Phelan (1982); in the present study, where the substantia nigra was confounded to HRP (WGA-HRP) was used combined with histochemical staining for acetylcholinesterase (AChE) to further examine the connections.

A projection from the caudate nuclei to the substantia nigra has previously been described by Wilson and Phelan (1982); in the present study, these projections were labeled retrogradely from the substantia nigra. These labeled nigral cells could be identified by the location of the border zone. In immunoperoxidase injection of WGA-HRP within the neostriatum rostral to the border zone reveals a narrow band of retrogradely labeled terminals within the substantia nigra. In addition to projections to the more caudal globus pallidus and substantia nigra, double labeling of some sections for AChE suggests the band of labeled terminals is within the AChE-rich stratum. Scattered retrogradely labeled cells are also seen within the field of labeled terminals, indicating that the few neurons in the border zone give rise to reciprocal projections to the main body of the striatum.

343.14 POTENTIAL EVIDENCE FOR A NEW SUBDIVISION IN THE NEOSTRIATUM OF THE RAT. S.Y. Shyu* and G.H. Penn. Department of Anatomy and Cell Biology, School of Medicine, East Carolina University, Greenville, NC 27858

In horizontal or parasagittal Nissl sections through the rat neostriatum a caudal band of densely packed medium-sized cells can be seen lying at the border separating the neostriatum from the globus pallidus. This cell group, referred to here as the "medial pallidal" or "field-III" in shape, is divided into a flattened plate, occupying almost the entire dorsomedial and medioventral part of the border with the external medullary lamina. It is broken into islands of cells by the perforating internal capsule fascicles.

A projection from the caudate nuclei to the substantia nigra has previously been described by Wilson and Phelan (1982); in the present study, these projections were labeled retrogradely from the substantia nigra. These labeled nigral cells could be identified by the location of the border zone. In immunoperoxidase injection of WGA-HRP within the neostriatum rostral to the border zone reveals a narrow band of retrogradely labeled terminals within the substantia nigra. In addition to projections to the more caudal globus pallidus and substantia nigra, double labeling of some sections for AChE suggests the band of labeled terminals is within the AChE-rich stratum. Scattered retrogradely labeled cells are also seen within the field of labeled terminals, indicating that the few neurons in the border zone give rise to reciprocal projections to the main body of the striatum.

Small injections of tracer within the border zone reveals (1) retrogradely labeled cells elsewhere in the striatum, (2) widespread anterogradely labeled terminals extending for 500 microns from the injection site but confined within the border zone, and (3) projections to the globus pallidus and substantia nigra.

These results indicate that a marker in this border zone may provide an easily identifiable anatomical marker for the anterograde tractography of regions of the basal ganglia.

343.15 PROJECTIONS OF VISUAL CORtical AREAS VIA THE NEOSTRIATUM TO THE GLOBUS PALLIDUS AND SUBSTANtIA nigra in the Rhesus Monkey. J.A. Slate, G.C. Wieglerd and S. Dasgupta. Laboratory of Neuropsychology, Rockefeller University, New York, NY 10021.

Injections of multiple anterograde and retrograde tracers were made into the tail and genu of the caudate nucleus or adjacent ventral putamen under physiological control in five rhesus monkeys. After caudate injections, labeled cells were found in both a large continuous region of cortex corresponding to the site of injection, and in several non-contiguous cortical regions. Injections in the rostral tail, the continuously labeled region included area TE and adjacent portions of TF, TR, TG, and, occasionally, area 35. After injections into the posterior tail and ventral genu, labeled region shifted posteriorly to the parietal, then to the ventral and lateral, and finally to the lateral and parietal cortex and thalamus, and adjacent area 23.

The non-contiguous areas labeled by nearly all injections included the principal sulcus/frontal eye field region, area 24, the superior temporal polar sensory area, and, more rarely, area 25. Thus, whereas certain temporal, occipital, and parietal cortical areas project bilaterally, the neostriatum projects to anteriorly, probably as originally shown by Kemp and Powell, prefrontal, anterior cingulate, and superior temporal motor areas have a wider distribution, consistent with more robust inputs.

Labeled cortical cells were mostly layer 5 but also in layer 6. Labeled subcortical cells were found in the bed nucleus of the stria terminalis, anterior thalamic nuclei, posterior thalamic nuclei, and substantia nigra pars reticulata. Whereas the ventral genu and entire tail of caudate and adjacent ventral portion of the globus pallidus at the border between its internal and external subdivisions, the dorsal genu and adjacent body of caudate projected to GP at its dorsal border. Both ventral and medial portions of the substantia nigra projected to the rostrocaudal 3Nl in a partially overlapping fashion.

Because of the known projections from both GP and SNl to the frontal lobe via the thalamus and from SNl to the superior colliculus, the neostriatum could provide alternate routes by which visual cortical areas could influence dorsolateral, frontal, and collicular regions to which they project directly. The latter regions are thought to have spatial and/or visuomotor functions, and, interestingly, that the portions of the caudate receiving visual inputs also receive inputs from polysensory areas implicated in these functions.


The ventral pallidum of the rat receives its input from the nucleus accumbens while the dorsal pallidum receives its input from the caudoputamen (Haber and Swanson, Trends Neurosci. 5,53). Enkephalin-positive cells are found throughout the globus pallidus and the ventral pallidum; substance P and calcitonin gene-related peptide are also found throughout the ventral pallidum and have been suggested as a marker for the ventral pallidum (Haber and Nauta, Neurosci. 9,245). The distribution of enkephalin, and substance P-positive cells were recently described in the globus pallidus and substantia nigra (Haber and Watson, Neurosci. 14,1011). Dense enkephalin-like immunoreactivity is observed throughout the external segment of the ventral pallidum. Substance P-like immunoreactivity is more limited in the pallidal region and is found in a thin semi-circular band ventral to the anterior commissure but not directly beneath it and extending dorsomedially into the rostral pole of the external segment. This pattern is similar to monkeys but unlike the ventral pallidum of the rat where substance P-positive fibers show a more extensive ventral subcommissural distribution but does not extend into the dorsal pallidum. It raises the question of what area in the primate forebrain is homologous with the ventral pallidum of the rat. From the staining patterns it might be inferred that the area defined as the dorsal pallidum in the rat and receiving input from the caudate nucleus rather than from the nucleus accumbens is, in primates larger, and extends beneath the anterior commissure, thereby pushing the ventral pallidum, or that region which receives input from the nucleus accumbens, ventralward as well as dorsomedially into the rostral pole of the external segment.

We report here the results of immunohistochemical study of the projections from the nucleus accumbens to the rhesus monkey with emphasis on the pallidal region. Methods: Control experiments involved the dorsal striatum. Preliminary findings indicate that the nucleus accumbens, in primates, projects to a region ventral to the anterior commissure but not directly beneath it. These terminal fields are also observed in the rostral pole of the external segment of the globus pallidus but are not found in more caudal regions of that segment. This projection appears to be reciprocal as labeled WGA-HRP cells are located among the terminal fields in these pallidal regions. This projection from the nucleus accumbens to the pallidum coincides well with the distribution of substance P-positive fibers.
BASAL GANGLIA III
FRIDAY PM

435.17 CELLS IN THE LATEROPosterior THALAMIC NUCLEUS AND SUBSTANtIA nigra Pars LATERALIS MAY INTEGRATE THE VISUAL SYSTEM WITH THE BASAL GANGLIA. Z.K. Li* and M. Takada. Dept. of Anatomy, Univ. of Toronto, Toronto, Ontario MSS 1A8 Canada.

The lateroposterior thalamic nucleus (LP) is the major source of visually related thalamostriatal projections. The LP sends fibers to the secondary visual cortex and in turn receives inputs from the secondary visual cortex and superior colliculus (SC) and further from the substantia nigra pars lateralis (SNL). In addition to its LP efferents, the SNL sends axons to the SC and amygdala and further to the striatum. These facts prompted morphological approaches to integrative mechanisms between the visual system and basal ganglia. The first goal of this study was to examine whether or not single LP neurons give rise to divergent collateral projections to the visual cortex and striatum, and whether or not single GL neurons give off axonal branches to the LP/SC and striatum. The data from our retrograde fluorescent double labeling experiments (with true blue and diamidino yellow) in the rat clearly show that a small population of LP cells projecting to the striatum have axon collaterals to the visual cortex, whereas a large population of single SNL cells project to both the LP/SC and striatum.

Furthermore, considerable histochemical studies have previously suggested that the SNL cells contain dopamine as a possible neurotransmitter. The second goal of our experiments was to identify if the SNL cells projecting to the LP, SC, striatum and/or amygdala contain dopamine. Employing a combined retrograde fluorescent tracing (with fluoro-gold) and immunofluorescent histochemistry for tyrosine hydroxylase, the presence of dopamine, was determined that subpopulations of SNL cells sending fibers to each target are presumably dopaminergic, although the proportion of dopaminergic cells in each group can vary from more than 80% (to the amygdala) to less than 10% (to the LP and SC).

In conclusion, SNL cells in the LP and SNL might provide an important role in integrating visual and motor systems.

(Supported by the Medical Research Council of Canada)

435.19 PALLIDAL PROJECTIONS TO SURTHALAMUS, SUBSTANTIA NIGRA AND PEDUNCULO-PONTINE NUCLEUS: EVIDENCE FOR COLATERALIZATION OF PALLIDAL PROJECTIONS REVEALED BY WHEAT GERM AGGLUTININ-HISTOCHEMISTRY AND NADPH-DIAPHORASE TOXIN B IMMUNOCHEMISTRY. S. Afsharpour, H. Kja, and S.T. Kjaer, Department of Neurology and Neuropathology, University of Copenhagen, Dept, of Neurology, Hvidovre Hosp., Toronto, ON, M5A 2K2, and Sch. of Med. and Edith Nourse, Rogers Hosp., VA Hospital, Bedford, MA 01730.

Excitotoxin models suggest that striatal degeneration in Huntington's disease (HD) may be related to glutamatergic corticostriatal input. Recent studies have shown that in HD aspiny neurons containing NADPH-diaphorase (NADPH-d), somatostatin, and neuropeptide Y (NPY) are selectively spared while spiny neurons are severely involved. Since dendritic spines are the major site of termination of corticostriatal afferents, the aspiny neurons may be spared because of a paucity of corticostriatal synapses. This is an electron microscopic study of NPY and NADPH-d containing neurons in the rhesus monkey and of their synapses with degenerating corticostriatal axon terminals.

Areas of frontal cortex were aspirated in several adult rhesus monkeys. After 5 days they were perfused and the ipsilateral basal ganglia were removed and vibratome-sectioned at 100 µm. Adjacent sections were stained with the final fixative method and with NADPH-d histochemistry of NPY immunocytochemistry. Labelled neurons located in zones of terminal degeneration as indicated by adjacent fluorescent sections. These were stained with or without electron microscopy. Extensive portions of these neurons were reconstructed to demonstrate synapses with degenerating corticostriatal axon terminals. Sonata receive several types of axon terminals making symmetrical or asymmetrical synapses. Dendrites also form both types of synapses. The asymmetrical type is far more frequent beyond the most proximal dendritic segments. Most of the identified neurons were located within neuropil containing degenerating corticostriatal axon terminals which formed asymmetrical synapses with spines, dendritic shafts and somata. Occasionally did these synapses involve the dendrites or somata of the identified neurons.

The survival of aspiny neurons in HD may, in part, be due to receiving few corticostriatal synapses. Quantitative analysis of the corticostriatal synapses of aspiny and spiny caudate neurons will be required to better assess this possibility.

Supported by the Neurology Faculty Development Fund of Emory University, NIH grant NS26941, and the Institute for Neurological Research Inc.
436.1 ORGANIZATION OF STRIATAL AND SUBTHALAMIC NUCLEUS AFFERENTS TO PALLIDUM IN PRIMATE AS REVEALED BY PHA-L ANTEROGRADE TRACING METHOD. J. Haradat, A. Parent, and Y. Smith. (SPHINX Boucher) Lab. of Neurobiology, Fac. of Med., Laval Univ., Québec, Canada. The lectin Phaeosolus vulgaris-leucoagglutinin (PHA-L) was used as an anterograde tracer to study the patterns of termination of striatopallidal and subthalamopallidal fibers in the squirrel monkey (Saimiri sciureus). Small iontophoretic injections of PHA-L in the subthalamic nucleus (ST) led to prominent fiber labeling in the ipsilateral globus pallidus (GP). The overall pattern of fiber segregation observed was similar to that seen after injection of the anterograde tracer Phaseolus vulgaris-leucoagglutinin (PHA-L). The majority of fibers were regrouped into two distinct bands lying along the rostrocaudal axis of typical medium-sized spiny neurons close to the injection site. These fibers arborized profusely within the internal medullary lamina where they displayed a typical band-like pattern. In addition, a small to moderate number of fibers arborized in all directions were present in the core of GPi and GPe. In the rostral third of GP, fibers were more numerous in the GPi than in GPe. Here, they formed two distinct bands; one in the central core, the other along the inner and outer surfaces of the internal medullary lamina. In the middle third of GP, fibers abounded along the medial pole of GPi where they arborized profusely in all directions. In the caudal third of GP, most labeled fibers occurred in the inner laminae (GPi) and extended rostrally throughout GP. In the rostral third of GPe, fibers were more numerous in the GPe than in GPi. Most labeled fibers swept laterally along the anseral laminae and invaded GP from its ventral surface. These fibers ascended within medullary laminae and arborized profusely within both internal (GPi) and external (GPe) pallidal segments. In the caudal third of GP, numerous thick and smooth TH fibers occurred in the dorsal portion of each segment, whereas they were absent in all directions. In the middle third of GP, TH fibers were significantly more numerous in GPi than in GPe. In GPi, they formed numerous small plexuses distributed over the entire dorsoventral extent of the segment, whereas GPe displayed only few short fibers that were uniformly scattered throughout its dorsal portion. In the rostral third of GP, TH fibers remained more abundant in GPi than in GPe, but the rostral pole of GPe contained a significant number of fibers forming small plexuses oriented dorsolaterally toward the putamen. Other TH fibers were seen to label the lateral hypothalamus to ascend along the lateral border of the reticular thalamic nucleus or to continue rostrally toward the olfactory tubercle. The results reveal that the dopaminergic innervation of the subthalamic nucleus in primates is relative weak and of unexplained rostrocaudal differences. In contrast, the pallidum receives a massive dopaminergic afferent arborizing primarily within its internal segment. (Supported by MRC and FRSQ).

436.2 DOPAMINERGIC INNERRATION OF PALLIDUM AND SUBTHALAMIC NUCLEUS IN PRIMATE. Y. Smith and A. Parent, Lab. of Neurobiology, Fac. of Med., Laval Univ., Québec, Canada. An antiserum raised against tyrosine hydroxylase (TH) was used to study the dopaminergic innervation of the basal ganglia in the squirrel monkey (Saimiri sciureus). Complete series of transverse and horizontal sections of midbrain and basal forebrain were incubated with this TH antiserum, whereas some alternate sections were also incubated with an antibody against tyrosine hydroxylase (OHB) antiserum. The dopaminergic nature of neuronal elements described previously in the midbrain-striatal pathway was confirmed in that they all stained positively for TH but not for OBH. Axons of midbrain TH-immunoreactive cell bodies accumulated mediodorsal to the substantia nigra pars compacta GPi and formed a massive bundle ascending through the field H of Forel. The dopaminergic innervation of the globus pallidus (GP) derived mostly from the substantia nigra pars compacta GPi and formed fascicular caudally and the area liminarius rostrally. Some TH fibers coursed along the lateral fascicularis fiber arborized within the dorsomedial area, whereas most traversed the internal capsule and invaded GP from its ventral surface. These fibers ascended within medullary laminae and arborized profusely within both internal (GPi) and external (GPe) pallidal segments. In the caudal third of GP, numerous thick and smooth TH fibers occurred in the dorsal portion of each segment, whereas they were absent in all directions. In the middle third of GP, TH fibers were significantly more numerous in GPi than in GPe. In GPi, they formed numerous small plexuses distributed over the entire dorsoventral extent of the segment, whereas GPe displayed only few short fibers that were uniformly scattered throughout its dorsal portion. In the rostral third of GP, TH fibers remained more abundant in GPi than in GPe, but the rostral pole of GPe contained a significant number of fibers forming small plexuses oriented dorsolaterally toward the putamen. Other TH fibers were seen to label the lateral hypothalamus to ascend along the lateral border of the reticular thalamic nucleus or to continue rostrally toward the olfactory tubercle. The results reveal that the dopaminergic innervation of the subthalamic nucleus in primates is relatively weak and of unexplained rostrocaudal differences. In contrast, the pallidum receives a massive dopaminergic afferent arborizing primarily within its internal segment. (Supported by MRC and FR-SQ).

436.3 THE TOPOGRAPHICAL EFFECTS OF NOMIFENSINE ON STRIATAL DOPAMINE AS MEASURED BY IN VIVO VOLTAMMETRY. J.E. Glynn* and B.K. Yamamoto (SP: B. Donzanti). Dep. of Pharmacology, Northeastern Ohio Univ. Coll. Med., Rootstown, Ohio 44272. Nomifensine is a dopamine uptake blocker that has been used to study the dopaminergic innervation of specific sites in the brain (Scatton et al., 1985) with no direct dopamine releasing properties. The aim of the present study is to further characterize the neurochemical heterogeneity of the striatum by establishing a functional topography of dopamine uptake in the caudate-putamen. Male Sprague-Dawley rats were anesthetized with urethane and implanted with stereotaxically-modified carbon fibers (200µm). In vivo voltammetric recordings using linear sweep scans from -0.15 to 0.4V and semidifferential signal processing were made at 5 min intervals. Peak currents were measured at 0.15V for dopamine. Nomifensine (10 mg/kg i.p.) was injected after a 30 min stable baseline. The resulting dopamine release in response to nomifensine was studied at different subregions. Male Sprague-Dawley rats were anesthetized with urethane and implanted with stereotaxically-modified carbon fibers (200µm). The probe was perfused with a modified Ringer's solution at a rate of 2µl/min. The dialysates were collected at 20 min intervals and analyzed for dopamine and DOPAC content by HPLC with electrochemical detection. After a 1 hr stable baseline period, rats were injected i.p. with the D2 antagonist (-)-Sulpiride (25 mg/kg) or d-amphetamine (2.5 mg/kg) and samples collected for a 4 hr period. All data for dopamine release are expressed as percent of baseline ± SEM (n=4/group). The dopamine release in response to d-amphetamine is presented as a percent of baseline ± SEM (n=4/group). The dopamine release in response to d-amphetamine is presented as a percent of baseline ± SEM (n=4/group). The dopamine release in response to d-amphetamine is presented as a percent of baseline ± SEM (n=4/group). The dopamine release in response to d-amphetamine is presented as a percent of baseline ± SEM (n=4/group).

436.4 REGIONAL DOPAMINE RELEASE IN RAT STRIATUM MEASURED BY IN VIVO MICRODIALYSIS. B.K. Yamamoto, Dept. of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272. We have previously reported a topography of dopamine release in cat striatum using in vivo dialysis (Neurosci. Abstr. 1986). The present study, using in vivo brain microdialysis, confirms and extends our previous findings. The aim of the present study is to further characterize the neurochemical heterogeneity of the striatum by establishing a functional topography of dopamine uptake in the caudate-putamen. Male Sprague-Dawley rats were anesthetized with urethane and implanted with stereotaxically-modified carbon fibers (200µm). The probe was perfused with a modified Ringer's solution at a rate of 2µl/min. The dialysates were collected at 20 min intervals and analyzed for dopamine and DOPAC content by HPLC with electrochemical detection. After a 1 hr stable baseline period, rats were injected i.p. with the D2 antagonist (-)-Sulpiride (25 mg/kg) or d-amphetamine (2.5 mg/kg) and samples collected for a 4 hr period. All data for dopamine release are expressed as percent of baseline ± SEM (n=4/group).
EVIDENCE FOR A HIGH PERCENTAGE OF DOPAMINERGIC, CROSSED MESOSTRIATAL PROJECTIONS IN RABBIT. D.M. Ripps, S.P. Ous, T.W. Baranz, (PHR R. Jennings) Dept. of Behavioral Neuroscience, Center for Neurochemistry, Univ. of Pittsburgh, Pittsburgh, PA 15260.

The mesostriatal projection has been studied extensively, and is considered to be primarily ipsilateral in rats. Only 0.5-2.0% of substantia nigra and ventral tegmental area (SN-VTA) neurons project to areas located contralaterally. We have studied the mesostriatal projection system in the rabbit using retrograde tracing techniques, and have found much higher percentage of contralateral projection. All animals were adult New Zealand white rabbits. Either single or repeated injections of 3%-3'-diaminobenzidine (DAB) was injected into the substantia nigra, and the fluorescent dye tritiated thymidine (3H-TM) or the fluorescent dye primuline (1%, 0.8 μl) was injected unilaterally into the caudate, and brain tissue was processed, respectively, with diaminobenzidine (DAB) and primuline. Labelled neurons throughout both sides of SN-VTA complex were stained using dark field microscopy for DAB-reacted HRP, and epi-illumination fluorescence for primuline; retrogradely transported primuline appears as yellow granules in the cytoplasm.

Consistent with previous studies, labelled neuronal somata in the contralateral SN and VTA were considerably fewer in number than seen ipsilaterally. In addition, labelled cells appeared to be located at sites corresponding to the center of clusters of ipsilaterally labelled neurons. However, the percentage of contralaterally labelled neurons was found to be 10-12%, significantly higher than reported previously for rats. We further extended previous study on the nature of the crossed projections by combining peroxidase-antiperoxidase or peroxidase-antiperoxidase for tyrosine hydroxylase with the above retrograde tracers. A combined method for visualizing 3H-DAB cobalt-stabilized HRP tracers simultaneously with DAB-labelled immunoreactive axons was devised. In the procedure one of the antigens was visualized by the DAB reaction, and the other by the peroxidase reaction. Neurons in SN-VTA with peroxidase-positive and red/purple pricipitate at immunoreactive sites, and thus, a different color spectrum fluorescence from the same neurons in immunostained tissue. This facilitated the identification of double labelling and reduces interference between the two labels. Preliminary data have shown a high percentage of projecting neurons both ipsilaterally and contralaterally simultaneously reported - Supported by NS13608.

Evidence for a monosynaptic relationship between dopaminergic neuron and cholinergic neurons in the rat striatum. A dual-labeling immunocytochemical study. I.L. Cheng, Department of Anatomy and Physiology, University of Memphis, Memphis, College of Medicine, The Health Science Center, Memphis, TN 38163.

The synaptic relationship between cholinergic neurons and dopaminergic axons in the striatum was examined by a dual-labeling immunocytochemical method. Cholinergic neurons were identified by their immunoreactivity for choline acetyltransferase (ChAT) and dopamine neurons by their immunoreactivity for tyrosine hydroxylase (TH). The dual-labeling method is based on the principle that dopaminergic axons are preferentially identified by their positive immunoreactivity for tyrosine hydroxylase (TH). Monoclonal anti-TH and rabbit anti-ChAT antibodies were kindly provided by F. Eckenstein, and G.D. Crawford and P.M. Salvaterra, respectively. Rabbit anti-Th antibodies were purchased from Immunex Corp. In order to simultaneously visualize and distinguish both antigens within the same tissue sections at either light or electron microscopic levels, dual labeling immunocytochemistry procedures were employed in which one of the antigens was labeled by HRP-DAB reaction products while the other was labeled with silver-intensified colloidal gold particles or silver-intensified HRP-DAB reaction products.

At the light microscopic level, large cholinergic neurons were found in both dorsal and ventral striatum. The long and sparsely branched dendrites of these cholinergic neurons were frequently cut at the surfaces of the 50 μm thick Vibratome sections. The surrounding striatal neuropil was densely innervated with fine, straight, well-preserved cholinergic fibers and terminals. Numerous TH-positive fibers and terminals were observed to form synapses with unlabeled, and possibly monoaminergic neurotransmitters. Identification of synapses between differentially labeled profiles was difficult because synaptic specializations were obscured by reaction products. However, a small number of close appositions of these synaptic contacts were found between TH-positive terminals and Chat-positive dendrites. This result suggests that dopaminergic axons terminals may have monoaminergic influence on cholinergic neurons in the striatum.

Supported by USPHS Grants NS21003 and AG05944 and a research grant from the University Physicians Foundation of the University of Tennessee, Memphis.
In a previous study using three-dimensional computer-assisted reconstructions, we demonstrated that the enkephalin-rich patches in the neostriatum of the adult cat are distributed in a highly organized pattern consisting of large finger-like structures radiating diagonally from the ventricular edge across the width of the caudate nucleus. These structures are connected via numerous smaller crossbridges to give the appearance of a lattice-like pattern of enkephalin immunoreactivity within the caudate nucleus.

In the present study, the 3-dimensional distribution of choline acetyltransferase (ChAT) containing perikarya in the cat neostriatum and their relationship to the enkephalin patches were investigated. Series 50 μm coronal sections were cut through the head and body of the caudate nucleus and stained alternately with TH and with anti-ENK antibodies. The location of individual ChAT-positive perikarya and the outlines of the enkephalin patches were digitized from camera lucida drawings and entered into an IBM-PC computer. Sections were aligned using the outline of the caudate and major blood vessels for registration. ChAT-positive perikarya were distributed throughout the neostriatum. In reported elsewhere, the majority of these neurons lie outside of the enkephalin patches. A preliminary observation of limited reconstructions suggests a similar pattern in the distribution of these cells similar to that revealed from three-dimensional reconstructions of enkephalin patches. Specifically, clusters of ChAT-positive perikarya appear to form diagonal striations which emanate from surface and radial surrounding areas containing few labelled perikarya. The relation of the thalamostriatal projection, organizational and cholinergic neurons to that of the enkephalin network may provide a framework for understanding information processing within the neostriatum.

This work was supported by the Office of Naval Research ONR N00014-85-N-0699, NIH AG05344 and MDA 0079 and 02854.


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PARVALBUMIN IS PRESENT IN GABA CONTAINING NEURONS IN THE SUBSTANTIA NIGRA OF THE CAT. L. Cowan, C. W. Wilson, and P. C. Zimmerman, Department of Anatomy and Neurobiology, Un. of Tennessee School of Medicine, 875 Montague-Memphis TN 38133. The present finding suggest that these neurons may also contain the calcium binding protein, CaBP. CaBP is known to be localized preferentially in Purkinje cells and granular cells. The present finding suggest that these neurons may also contain the calcium binding protein, CaBP. CaBP is known to be localized preferentially in Purkinje cells and granular cells.

This study demonstrates directly that virtually all nigral neurons in cat contain the neurotransmitter GABA and are likely to have an inhibitory action on the superior colliculus, as previously demonstrated in other species. SNL, SNR, and SNC were noted as separate projections to SC. The nigral pathway in cat is therefore more complex than is sometimes recognized. Supported by USPHS grant EY-02973-07.

Supported by grant NS 20743 and RCDA NS 01078 from NIH.
436.13 THE EXPRESSION OF SOMATOSTATIN AND SUBSTANCE P IMMUNOREACTIVITY IN PRIMARY CULTURES OF RAT NEUROTROPHS. E. Gallarraga*, B.J. Sumpter, and S.T. Kitai. Dept. of Anatomy & Neurobiology, College of Medicine, University of Tennessee, Memphis, The Health Science Center, Memphis, TN 38133.

Two largely separate populations of neuuropeptide-containing striatongrial projection neurons have been distinguished in pigeons, one population whose neurons contain substance P (SP) and dynorphin (DYN) and a second population whose neurons contain enkephalin (ENK) (Reiner, Brain Res., ‘86). In the present study we investigated the development of neurons expressing SOM and SP immunoreactivity in monolayer neonatal cultures prepared from embryonic rat striatum as previously described (Kitai, et al., Neurosci. Abstr. 12., 655, 1986).

Studies were performed on neurons in culture 4 to 27 days. For immunocytochemistry, cultures were fixed with 4% paraformaldehyde, 0.2% glutaraldehyde solution. Following treatment with 0.25% triton X-100, cultures were incubated with either SOM or SP antisera (dilution 1:100 in sodium PBS with 2% normal goat serum for SOM; 1:100 in sodium PBS with 2% normal rabbit serum for SP) for 3 hrs at 4°C. After rinsing, the tissue was incubated for 1 hr, with biotinylated anti-rabbit IgG for SOM and anti-rat IgG for SP (1:100 in sodium PBS with 0.2% triton X-100 and the respective normal serum. Immunoreactive neurons were localized with the biotin-avidin procedure. The HRP-antibody complex was visualized with the diaminobenzidine-glucose oxidase technique.

Specific staining for SOM and SP was seen in all ages studied. Immunostaining for SOM was seen mainly to soma and, in the case of SOM, to some proximal processes. The size and shape of immunoreactive cells in the case of both these cells was unipolar (80% SOM+, 86% SP-IR), but bipolar (17.5% SOM+, 88% SP-IR) and multipolar (2.5% SOM-, 4% SP-IR) cells were also stained. Our results indicate that the proportion of SOM cells increased with culture age: from 19% (4 DIV) cultures to 30% (5 DIV) at 5 DIV. In contrast, the proportion of SP-IR neurons increased from 5% (DIV) to 15% at 3 DIV, but remained steady thereafter. No evidence for induction or delayed-expression of either phenotype was observed. Ages in proportion, SP-IR neurons decrease in number more rapidly than SOM-IR neurons.

This suggests long term survival in pure striatal culture could be favor SOM staining.

Supported by NIH Grant NS-20702 to S.T.K.

436.14 STRIATONGIAL PROJECTION NEURONS: A RETROGRADE LABELING STUDY OF THE RELATIVE NUMBERS THAT CONTAIN SUBSTANCE P OR ENKEPHALIN. C.D. Anderson and A. Reiner, Dept. of Anatomy & Cell Biology, Univ. of Michigan, Ann Arbor, MI 48109.

Two largely separate populations of neuromodulator-containing striatongrial projection neurons have been distinguished in pigeons, one population whose neurons contain substance P (SP) and dynorphin (DYN) and a second population whose neurons contain enkephalin (ENK) (Reiner, Brain Res., ‘86). In the present study we investigated the development of neurons expressing SOM and SP immunoreactivity in monolayer neonatal cultures prepared from embryonic rat striatum as previously described (Kitai, et al., Neurosci. Abstr. 12., 655, 1986).

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Supported by NIH Grant NS-20702 to S.T.K.
neuronal migration between aggregates, however, continued for at
least 2 weeks after plating. These results suggest that striatal
compartmental boundaries are intrinsic and determined by the
primary tissue culture model of the developing brain has
been studied in detail with immunocytochemical methods employing
antibodies against DA. Rat embryos, foetuses, pups and adults were
studied, and the presence of cholinergic and somatostatin (SOM)-containing
neurons was determined by analyzing the proportion of neurons double labelled with the 
antigen and tritium among all immunolabelled neurons, as a function of the 
day of thymidine injection.

The majority of CHAT-immunoreactive neurones in the striatum
became post-mitotic on embryonic (E) day 12-13, although there was a
caudal to rostral gradient in cholinergic neurogenesis. At caudal levels of the striatum, 
the peak of final mitotic activity occurred on E12. At intermediate levels, most CHAT-positive
neurons underwent their final mitotic division on E15 and E16. 
CHAT-positive neurones at the most rostral levels of the striatum
became post-mitotic on days E12 to E15. In contrast, the neurogenesis of SOM-immunoreactive neurones
was different in that of the CHAT-positive interneurons, mostly on E15 and E16, 
irrespective of the level within the striatum. The neurogenesis of both CHAT- and SOM-positive neurones was
accelerated and fast in the ventral striatum, in contrast to the slow
neurogenesis in the dorsolateral striatum. Therefore, a quantitative analysis of neuronal migration was
attempted using primary dissociated cultures of embryonic
eventually reach the substantia nigra earlier during
differentiation than neurons of the matrix compartment By injecting
retrograde tracer into the substantia nigra of E18 rat embryos, we were
able to detect catecholaminergic projections that originated in the
dorsal striatal regions. To test the hypothesis that DA plays an
organizational role in the development of the striatum, the ontogeny of the DA system
was studied in detail with immunochemical and autoradiographic methods. The presence and
distribution of dopamine (DA) could be detected as early as E10. The number of
neurochemically identified neurons was determined by analyzing
the proportion of neurons double labelled with the antigen and 
tritium among all immunolabelled neurons, as a function of the
day of thymidine injection.

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TESTOSTERONE REGULATES THE SUBSTANCE P INNERVATION OF THE PEPTIDES: ANATOMICAL LOCALIZATION IV

FRIDAY PM

MALE GOLDEN HARMSPT, SUANZO, AND NIMH grant MH 33127.

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The central distribution of the nonpeptide oxytocin may account for its effects on behaviors as maternal and sexual behavior. The majority of this steroid affected the STS nucleus of the amygdala, where neurons are located. The presence of neurons in the STS nucleus of the amygdala (M) is known to be involved in maternal behavior. The ovarian steroids affecting these behaviors influence pregnancy and postpartum in mice by examining serial brain sections and not associated with blood vessels in lactating animals. Whereas lactating animals may influence postpartum behaviors such as oxytocin neurons perhaps supplement the high levels of oxytocin topography in ovariectomized control mice. While perivascular topography of mothers after weaning was comparable to the area was not as small as in normal females (mean 4.65 sq. mm). Immunoperoxidase was visualized with the peroxidase-anti-peroxidase method. Immunostained perikarya were visible in all experimental groups in the STS nucleus as well as in various accessory nuclei.

Unlike cells in the magnocellular nuclear, that showed no changes across pregnancy and parturitum, oxytocinergic neurons in "accessory" nuclei showed varying topography across these states. Oxytocinergic cells in the lateral subcoeruleal nucleus, preoptic region and the perifornical region were clearly clustered around blood vessels in late pregnancy and postpartum. Cells in these same areas and perivascular scattered cells were scattered and not associated with blood vessels in lactating animals. Whereas these clustered cells sent their projections along blood vessels scattered cells seen in lactating animals clearly sent projections through the neuropli to central sites. Oxytocinergic brain topography of mothers after weaning was comparable to the topography in ovariectomized control mice. While perivascular oxytocin neurons perhaps supplement the high levels of oxytocin necessary peripartum, centrally projecting oxytocin neurons in lactating animals may influence postpartum behaviors such as maternal behavior.

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EFFEUCT OF CASTRATION AND TESTOSTERONE TREATMENT ON SUBSTANCE P LEVELS WITHIN THE VOMERONASAL PATHWAY OF THE MALE GOLDEN HARMSPT PATHWAY OF THE

UNITED STATES: ANATOMICAL LOCALIZATION IV

FRIDAY PM

Male mating behavior in most vertebrate species is dependent on circulating gonadal steroids. While it is clear that steroids regulate mating behavior through action on the CNS the neurochemical mechanisms which mediate steroidal action are poorly understood. Mating behavior in the male golden hamster is regulated by three critical behaviors: mating, sexual behavior, and mounting behavior. It has been previously demonstrated changes in oxytocin immunoreactive levels during late pregnancy and parturition (Caldwell et al. Neuroendocrinology, 1984, 39:231). We hypothesized that the high levels of oxytocin in its area were regulated by T. The gradual decline in sexual behavior which follows castration is interesting, as it parallels the gradual decline in its size following castration is interesting, as it

The medial nuc. of the amygdala (M) is known to be involved in the control of gonadotropic hormone release and in the control of reproductive behavior (matting, aggregation behavior). The most caudal part of M stains very darkly for substance P in both sexes, but the area of this dense SP staining in M was reduced at 10 days post-castration. We thus, there is probably a differential innervation of tissues in the reproductive tract by nerves containing one vs. more than one neurotransmitter substance (NPY or NPY + NA) innervate myometrial smooth muscle and if fibers innervating the reproductive tract by nerves containing one vs. more than one neurotransmitter substance (NPY or NPY + NA).

The medial subdivision, w ith the highest concentration in the dorsal parts of the postcommisural BNSTm is medially to the stria terminalis in the M. In the M the highest concentration of neurons containing SP-IR was located in the medial subdivision with a dense accumulation of fibers and cells in the caudal parts. The highest concentration of cells and fibers in the caudal parts. The highest concentration of neurons containing SP-IR was located in the medial subdivision with a dense accumulation of fibers and cells in the caudal parts.

Thus, there is probably a differential innervation of tissues in the reproductive tract by nerves containing one vs. more than one neurotransmitter substance (NPY or NPY + NA).

In some organs, e.g. gut and gut, it has been demonstrated that nerves chemically coded for a certain transmitter, or combination of transmitters, may be recruited to con find a neurotransmitter substance (NPY or NPY + NA). It is becoming established that nerve fibers of many tissues and organs may co-store two (or more) neurotransmitter substances. In some organs, e.g. gut and gut, it has been demonstrated that nerves chemically coded for a certain transmitter, or combination of transmitters, may be recruited to con find a neurotransmitter substance (NPY or NPY + NA)....

Nerve fibers in the guinea-pig ovary display calcitonin gene-related peptide (CGRP)-like immunoreactivity (Udman et al. Regul. Pepti.des 15:1, 1986). The present study investigated 1) the presence and distribution of CGRP-immunoreactive nerve fibers in the immature rat ovary, 2) the route by which these fibers enter the ovary, and 3) the potential effects of CGRP on steroid hormone production. Ovaries from intact rats, many CGRP-labeled fibers surrounded the ovary is unknown, but it may be more related to the control of blood flow than to the direct regulation of steroidogenesis.

Female Sprague-Dawley rats (28-30 days of age) were perfused with 4% paraformaldehyde, 0.1 M DL-lysine HCl and 0.1M sodium periodate. Additional ovaries were fixed by immersion in Zenobi's fixative. Other animals were subjected to transsection of the abdominal vagus nerves (AV), superior ovarian nerves (SON) or paraxial nerves (PN), or both the SON and PN at 24 days and sacrificed 6 days later. Ovaries were sectioned and processed for indirect immunofluorescence using antisera against [Tyr7] CGRP (22-27) at a dilution of 1:8000 (kindly provided by Dr. C. Sternini). Fluorescein-labeled secondary antisera was used at 1:100. Preabsorption of the antisera with 1μM synthetic CGRP blocked all specific staining. In ovaries from intact rats, many CGRP-labeled fibers surrounded blood vessels, although additional fibers coursed through the interstitial tissue. The AV or SON had no discernible effect on CGRP staining. In contrast, sectioning of the PN or combined SON and PN section eliminated all CGRP-like immunoreactivity in 5 of the 6 ovaries examined for each condition. In one ovary from each of these groups, a small number of labeled fibers were observed. CGRP, at doses ranging from 10^-10 to 10^-6 M, failed to alter ER2 and IP secretion from whole ovaries incubated in vitro or from granulosa cells of hypophysectomized, estrogen treated rats in culture. The peptide also failed to affect the E2 and P secretion from the granulosa cells of hypophysectomized, estrogen treated rats in culture. The results demonstrate that CGRP-immunoreactive fibers enter the ovary via the PN and are distributed predominantly with the vasculature. The function of CGRP in the immature ovary is unknown, but it may be more related to the control of blood flow than to the direct regulation of steroidogenesis. Supported by NIH HD-1731 and HD-02727, NSF BNS-8319017 and March of Dimes 5-254.

437.6 IMMUNOCYTOCHEMICAL EVIDENCE FOR A LAMPREY-LIKE GONADOTROPIN-RELEASING HORMONE GENE IN THE HUMAN PLACENTA.


Human placenta is a rich source of several hypothalamic and pituitary hormones and ovarian steroidogenic cells. Placental lamprey releasing hormone (LRH) concentrations were found to rise progressively during pregnancy. After parturition plasma LRH levels decreased rapidly within hours. Human term placenta homogenates contain large amounts of LRH and we have shown that human term placenta fragments secrete LRH in vitro. The bulk of placenta and circulating LRH in pregnancy exhibit the same apparent Mw as synthetic human LRH.

To investigate the origin of the high plasma levels of LRH in pregnancy, we first searched for the possible expression of the LRH gene in human term placentas. Placental RNA was extracted by the guanidine thiocyanate-cesium chloride technique. FL- and VP-immunoreactive fibers and VIP-immunoreactive fibers were found in paravertebral ganglia. In the present study was undertaken to test the hypothesis that a non-human form of LRH may be conserved during evolution and found in human brain. Immunocytochemistry was used to examine human tissues obtained within 6-18 hours postmortem. Two antisera generated to rat-MLH (1076, C811875) and two antisera generated to 1-GnRH (1467, 1459) were used in these studies. Antisera generated to 1-GnRH (1467, 1459) demonstrated less than 0.01% and 0.02%, cross reactivity, respectively, with m-GnRH. To eliminate the possibility of cross-reactivity in these immunocytochemical studies, the diluted antisera were incubated with mammalian LRH at a concentration of 10^-7 M at 4 °C overnight. The hypothalami was fixed by immersion for 24 hours in 10% acrolein in 0.1M phosphate buffer (pH 7.2). The murine and human technical procedures used have been described in detail (King, et al., J. Clin. Endocrinol. Metab. 50:88,1985). Neuronal cell bodies and fibers, immunoreactive with anti-antidromic reactivity was present in sections treated with lamprey-directed antisera (1467, 1459) and with a similar antigen excess (10^-7 M) of antiserum had no effect on the immunocytochemical reaction, and yielded results that were comparable to the non-absorbed lamprey antiserum. No immunoreactivity was present in sections treated with mammalian directed antiserum. In 10 women, 10 serum antisera (1467 and 1459) were not confined to specific nuclei, but were dispersed throughout the preoptic area and basal hypothalamus in all brains (2 female and 8 male). These immunoreactive neurons were primarily bipolar and were considerably less numerous than those seen on comparable to the non-absorbed lamprey antisera. No immunoreactivity was present in sections treated with lamprey-directed antiserum 1467, but with lamprey-directed antiserum (1459) that were incubated with synthetic 1-GnRH (10^-7 M) at 4 °C overnight, were dispersed throughout the preoptic area and basal hypothalamus in all brains (2 female and 8 male). These immunoreactive neurons were primarily bipolar and were considerably less numerous than those seen on comparable to the non-absorbed lamprey antiserum. No immunoreactivity was present in sections treated with mammalian directed antiserum (1459) that were incubated with synthetic 1-GnRH (10^-7 M) at 4 °C overnight. Our findings indicate that lamprey-like GnRH can be found in human hypothalamus and median eminence, supporting a possible role in the regulation of pituitary function. [K1A00296; HD19803; Great Lake Fish. Comm., NS: DCB-8602907, Brain Res 99/09/35]
437.9 NEURAL PERIKARYA IMMUNOREACTIVE WITH LAPMY LHRH ANTISERUM PROJECT TO THE NEUROHYPOPHYSEAL SYSTEM IN THE BRAIN OF PETROMYON MARTIUS, S. A. Silver, S. L. P. Anthony and J. L. King, Dep. of Anatomy, University of Alabama at Huntsville, Huntsville, AL, 35824; Dept. of Biology, Rhode Island College, Providence, RI, 02912; Dept. of Anatomy and Cellular Biology, Tufts University School of Medicine, Boston MA, 02111.

The role of LHRH in mammalian reproduction has been studied extensively; however, the role of the LHRH system in birds is not well characterized. In this study, we examined immunoreactive perikarya and fibers in the brain of the Hawaiian petrel, Pterodroma macrocephalus. Using the unlabeled peroxidase antiperoxidase method, immunoreactive perikarya and fibers with an antisera generated to lamprey LHRH, were also immunoreactive with certain antisera generated to mammalian LHRH. Immunoreactive perikarya were detected in an area of the hypothalamus identified as the mammillary nucleus. Immunoreactive fibers were confined to the dorsolateral areas of the hypothalamus. In contrast, no immunoreactive fibers were detected with a C-terminal extension antisera generated to the mammalian LHRH. These results are consistent with the hypothesis that the LHRH system in birds is evolutionarily conserved.


As a consequence of exposure to short photoperiods, reproductive development is markedly delayed in the male Djungarian hamster. Changes in the distribution or number of GnRH neurons are hypothesized to account for the inhibition of gonadotropin secretion and reproductive development associated with short photoperiods. In the present study, the distribution of GnRH neurons in the brains of postpubertal males was compared to that in hamsters with short photoperiod-induced delayed puberty. In hamsters aged 10 days of age, hamsters reared either in long days (16L:8D; n=4) or short days (8L:16D; n=4) were examined. Immunocytochemical staining of GnRH neurons was performed in male hamsters in which photoperiod-induced delayed puberty is associated with suppressed development of the GnRH neural system, and specifically, with decreased GnRH immunoreactive processes. These observations support the concept that hypothalamic regulation of GnRH neuronal activity is influenced by photoperiodic signals.

The distribution of NPY-like immunoreactive product within the hypothalamus was investigated in 6 monkeys ranging in age from 1 to 19 months. The monkeys were deeply anesthetized and perfused with saline, followed by a mixture of 45% paraformaldehyde and 0.1% glutaraldehyde in phosphate buffer, and were postfixed. The brains were cut in a cryostate in the transverse plane at 40 μm. Every fifth section was incubated in rabbit antisera to porcine NPY (NPY-F3; McDonald) and processed for PAP histochemistry. Adjacent sections were stained with cresyl violet acetate to identify cytoarchitectonic boundaries and the contours of the rhesus monkey hypothalamus by R. B. Blitzer (The University of Wisconsin Press, 1984).

Immunoreactive somata occurred in two locations: the bed nucleus of the stria terminals contained few stained somata whereas the arcuate nucleus displayed numerous labeled cells. In contrast to a previous finding in the squirrel monkey (Smith et al., J. Comp. Neurol., 236:71, 1985), no labeled somata were observed in the supraoptic and paraventricular nucleus of the rhesus monkey.

Immunoreactive varicose axons were distributed throughout the hypothalamus, but their concentration varied markedly. The smallest number of labeled axons was located in the anterior portion of the supraoptic nucleus and the various subnuclei of the mammillary body. A moderate number of fibers were seen in the lamina terminals, anterior, lateral, posterior, and dorsal hypothalamic areas, in the subventricular, suprachiasmatic, and ventromedial nuclei, and in the infundibular stalk. The largest concentration of varicose immunoreactive axons and punctate label occurred in the bed nucleus of the stria terminals, in the medial preoptic area, in the ventromedial, paraventricular, arcuate, and perifornical nuclei. In instances, particularly in the lateral hypothalamus, punctate label appeared along non-reactive dendrites and somata, suggesting synaptic contact between pre synaptic NPY-positive axons and unlabeled processes and cell bodies. Numerous immunoreactive fibers penetrate the ependymal lining of the third ventricle suggesting that these fibers either release NPY directly in the CSF to affect distant targets.

In view of these anatomical results and previous reports which indicate central effects of NPY on the secretion of luteinizing hormone, growth hormone and insulin in rats (McDonald et al., PNAS, 82:561, 1985; Nales 6:1155, 1985), we speculate that NPY may play a significant role in the hypothalamic regulation of hormone secretion in primates. (NIH grants RR-00165, EH-00001, HK-19731, HO-00792, and The March of Dimes 5-524.)


Oxytocin immunostaining of cells in the medial preoptic area (MPOA) has been described previously by ourselves as well as others. In the present study, by use of in situ hybridization and immunohistochemistry was used to investigate synthesis of oxytocin mRNA by cells in the MPOA and other regions of the rat brain.

Five micro frozen sections were prepared from 45 paraformaldehyde-fixed female rat brains. In situ hybridization was performed essentially as described by Han et al. (J. Neurosci. Res. 16: 97). The probe was a 32P-labeled synthetic oligodeoxyribonucleotide (38mer) complementary to the last 36 bases of exon C in the first two bases of the 3' untranslated region of the oxytocin mRNA.

Within the classical magnocellular nuclei, hybridizing cells were detected in the core of the suprachiasmatic nucleus, to the putative suprachiasmatic nuclei, and in the ventrolateral hypothalamus. Most hybridizing cells were also detected in the periventricular region, ventral and lateral to the fornix at the level of the hypothalamic thalamic extension. The hybridization signal was greatly enhanced by RNase pretreatment, and completely abolished by prehybridization with excess cold probe. In all hypothalamic regions where hybridizing cells were detected, vasopressin immunostaining cells were also detected in sections hybridized sections. We are currently using a combined immunohistochemical and in situ hybridization procedure on vibratome sections to confirm the presence of both oxytocin peptide and mRNA in the same cells in the MPOA, periventricular region, and other hypothalamic nuclei.

This work was supported by NIH grants NS00914, 1610150, NS 23040 and XHDR grant MH 33127.


Immunohistochemical studies have reported that opioid met-enkephalin (met-enk) appears to be co-localized with oxytocin in the hypothalamic magnocellular neurons as well as in the nerve terminals of the neurohypophysis. In addition, functional studies have indicated that met-enk inhibits the release of oxytocin from the nerve terminals of the neurohypophysis. Our previous studies have demonstrated that opioid octapeptide met-5-enkephalin-arg6-gly7-leu8 (met-enk-arg-gly-leu), which is derived from the precursor molecule proenkephalin A, was widely distributed in the magnocellular neurons, with distribution parallel to that of vasopressin in the hypothalamus of the rat. (Wang & Wyatt, Soc. for Neurosci. Abstr., 1, 1986). The aim of the present study, by use of immunohistochemistry, is to investigate the co-existence of met-enk-arg-gly-leu with pituitary hormones, oxytocin and vasopressin, in the hypothalamic neurons.

The rats were injected with colchicine (50 μg in 25 μl of saline) into the lateral cerebral ventricle before they were perfused. The brains were cut in a cryostat at 5-10 μm. The tissue sections were processed by the indirect immunofluorescence technique. The primary antisera were used at a dilution of 1:200 to 1:500. The sections were processed by the indirect immunofluorescence technique. The primary antisera were used at a dilution of 1:200 to 1:500. The antisera were pre-incubated with the corresponding synthetic peptides (Phe-Met-Arg-Phe-NH2 (FMRF-NH2) were recently isolated from bovine brain extract (Yang et al., Proc.Natl.Acad. Sci.USA 82:7757-7761, 1985). These peptides have an attenuating effect on morphine-induced analgesia when injected intracerebroventricularly in rats. Antiseras against the two peptides, an octapeptide Phe-Met-Arg-Phe-NH2 and a tetrapeptide Phe-Met-Arg-Phe-NH2, were raised in rabbits and characterized with standard radioimmunoassay, immunohistochemical binding controls and multiple experiments with synthetic peptides on nitrocellulose filter.

Two peptides that cross-react with an antiserum against the mol-
LOCALIZATION OF LUTEINIZING HORMONE RELEASING HORMONE (LHRH) IN THE PORCINE HYPOTHALAMUS BY IMMUNOCHEMISTRY.


Densely packed fibers were located in the ventral medial basal ventricular wall appeared to extend dorsal-ventrally toward the hypothalamus and median eminence. These initial findings suggest that the hypothalamus and median eminence. LHRH fibers along the third ventricle formed a compact bundle which coursed obliquely from clusters of ganglion cells of the nervus terminalis of the neonate. LHRH-reactive fibers were seen in these regions and were accompanied by thick bundles into the septum. From the triangular nucleus of the septum, these LHRH fibers ran down and around the rostral face of the brain, anterior to the nervus terminalis and lateral to the olfactory tubercle. An unusual observation was the apparent absence of LHRH cells in the hypothalamus in both the adult and neonatal gray opossum similar to that seen in several species of placental mammals.

Two antibodies (Dr. Ramirez CR11B73 and Mils-Teda 01010) yielded positive staining. Bipolar and multipolar LHRH perikarya were identified throughout the ventral septal-hypothalamic area, but were more densely packed in the area of the suprasylvian nucleus and the medial basal hypothalamus: predominantly the arcuate nucleus. Some perikarya were closely associated with the third ventricle in the area of the periventricular nucleus. Fibers containing LHRH were found from the ventral septal-hypothalamic area to the mamillary region. Dense-packed fibers were located in the ventral medial basal hypothalamus and median eminence. LHRH fibers along the third ventricular wall appeared to extend dorsal-ventrally toward the median eminence. The initial findings suggest that the distribution of LHRH staining within the ventral hypothalamus differs in detail from those reported for other large domestic species (ovine, bovine and equine).

The brains of 8 adult, and whole heads of 18 neonatal (days 0-12) gray opossums were fixed in Bouin's solution, embedded in Paraplast and serial 6μm sections cut and mounted onto glass slides. The immunocytochemical localization of luteinizing hormone-releasing hormone (LHRH) and luteinizing hormone (LH) was carried out with unlabeled antibody enzyme procedures. Localization with antisera to LHRH (CR1, Dr. Robert Benoit; 653, Dr. Lothar Jennes) revealed a distribution of LHRH in cells and fibers in the brain and in ganglia of the nervus terminalis of the adult and neonatal gray opossum similar to that seen in several species of placental mammals. LHRH-containing cells and fibers were seen in the medial septal nuclei, nucleus and tract of the diagonal band and olfactory tubercle. An unusual observation was the apparent absence of LHRH cells in the hypothalamus in both the adult and neonate. LHRH-reactive fibers were seen in these regions and were numerous in the median eminence. The nervous terminals in the hypothalamus showed several distinctive characteristics. Immunoreactive and non-reactive cells, observed in ganglia along the peripheral and intracranial course of this nerve, were accompanied by thick fascicles of LHRH fibers adjacent to blood vessels. The LHRH-reactive fibers in the central roots of this nerve formed a compact bundle which coursed obliquely from clusters of ganglion cells on the ventromedial surface of the olfactory bulbs into the septum. From the triangular nucleus of the septum, these LHRH fibers ran down and around the rostral face of the brain and the anterior commissure and fanned out into the medial preoptic area. As previously observed in the adult rat and guinea pig, LHRH fibers were detected in ganglion cells of the nervous terminals of the newborn gray opossum, preceding its detection in any other area of the brain of the newborn. LHRH fibers were detected in the anterior commissure and in the anterior hypothalamus. The presence of LHRH in the nervous terminals in the hypothalamus may be due to rapid synthesis and release of the neuropeptide. These results suggest that the brain LHRH system may function differently in the DS and DR rats and that the brain All system may function differently in the DS and DR rats. This work was supported by NSF BNS 8617117 and Amer. Heart Assoc. (IL) grants to J.A.W.
that the immune system is not mediated by the sympathetic nervous system. We therefore evaluated the effect of sympathectomy (a stress induced cataract lesion in the lateral septal area) on the immune response of female rats. In two experiments, we have tested the effects of electrolytic and KA lesions in the lateral septal area of female rats and have compared the effects of KA lesions in the lateral septal area with KA lesions in the hippocampal region on antibody production following immunization with 100 μg ovalbumin in complete Freund's adjuvant. Blood samples were collected on days 0, 7, 14, and 28 following immunization and on days 0, 1, 7, 14, and 28 in the second study. All animals were boosted with ovalbumin on day 14. The IgG, IgH, and IgG antibody titers were then measured by an enzyme-amplified ELISA assay. KA lesions in the lateral septal area reduced IgG titers on days 7, 14, and 17 with a trend for day 14. Similarly, the IgA and IgM titers were significantly lower on days 7, 14, and 17 for rats with KA lesions in the lateral septal area. In addition, the KA septal lesioned rats showed significantly elevated IgG titers on days 7 and 17 with a trend for day 14. These results suggest that neuroendocrine and immune regulatory processes are modulated by a common control system. Supported by MRC of Canada.

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Among the pathophysiological conditions observed following exposure to stress, it has been shown that stress alters the normal functioning of the immune and endocrine systems and produces gastrointestinal (GI) erosions. The studies reported here are the initial experiments in a series of investigations aimed at assessing a possible link between stress-induced GI erosions and alterations in immune function. Three chronic stress regimens were compared for effects on the integrity of the GI mucosa and the immune system. Since glucocorticoids have important immunomodulatory effects, we studied the role of stress-induced GI erosions, changes in plasma corticosterone levels were also measured.

Male Sprague-Dawley rats were exposed to one of the following: immobilization stress (IMM; 3 h/day for 10 days), performance stress (PS; avoidance or escape of electric footshock dependent upon performance of a task, 24 h/day for 14 days, Anderson et al., Pharmacol Biochem Behav, Relat: 829-833, 1987) and activity wheel stress (AWS; access to food limited to 1 h/day, continuous access to activity wheel for 7 days, Manning et al., Physiol. Behav, 21: 269-274, 1979). IMM was characterized by a moderate reduction in spleen size (1.6 ± 0.1 vs 2.3 ± 0.2 mg/blood weight for control rats) and thymus size (1.4 ± 0.1 vs 2.1 ± 0.3 mg/blood weight for controls). However, there were no changes in the proliferative responses to mitogenic stimuli (Concanavalin A, 0.25 or 2.0 μg/ml) of splenocytes from IMM rats. No significant changes in spleen or thymus size or lymphocyte proliferation were found in PS rats. In contrast, AWS produced a significant reduction in mitogen-stimulated lymphocyte proliferation (e.g., 6908 ± 4057 cpn at 1048 ± 11911 cpn for control at 0.5 μg/ml Con A. Spleen (1.3 ± 0.2 vs 2.6 ± 0.1 mg/blood weight for control) and thymus (0.6 ± 0.2 vs 1.7 ± 0.2 mg/blood weight for control) were also reduced by AWS. The immunosuppressive effects of AWS were most prominent in animals demonstrating a decline in running activity, body weight and food intake. There was also a pronounced increase in the incidence of GI erosions in AWS animals (21 vs. 8.6% vs 0.1 ± 0.1 lesions/stomach in controls), while gastric morphology was unaffected by AWS. These results suggest that significant degree of duodenal erosion with any of the stress regimens. However, among these AWS animals in which small duodenal lesions were found, the greatest degree of immunosuppression was evident. Circulating corticosterone levels were increased with IMM (338%) and AWS (231%) relative to control, but were not changed with PS. These results indicate that of the three chronic stress regimens tested, only the IMM regimen affects changes in immune competence, certain neuroendocrine parameters and the integrity of the gastric mucosa. Furthermore, these effects were not directly correlated with altered corticosterone levels. Thus, the AWS model appears to be suitable for assessing a possible link between stress-induced immunosuppression and erosion of the GI lining. (Work supported in part by a National Research Council Research Associateship to H.B.)
438.5 STRESSOR ASSOCIATED CUES INFLUENCE IMMUNE FUNCTION, J. Irvin, S. Livnat, and R. Adler, Queen's University, Kingston, Canada, K7L 3N6 and University of Rochester, Rochester, N.Y., 14620.

A variety of stressors have been shown to reduce the effectiveness of which the immune system defends the host from disease. For example, a number of laboratories have demonstrated that lymphocyte proliferation is inhibited following exposure to inescapable shock, cold, and other physical stressors. Similar findings have been made with Natural Killer (NK) cell lytic activity was attenuated in mice exposed to inescapable footshock or tailshock. The results of the present series of experiments suggest that the extent to which these stressors influence NK activity may depend on the environmental context in which the stressor is administered.

In an initial experiment, mice (C57BL/6J) were individually placed in shock boxes and exposed to one of two inescapable footshock protocols during a 1 hour period. Control animals were placed in adjacent boxes, but shock was withheld. Groups were subdivided and animals were sacrificed either immediately or 24 hours later for determination of splenic NK activity. Exposure to footshock did not significantly reduce NK activity in C3H/HeJ mice. Surprisingly, however, in the unshocked mice, NK activity was markedly increased 24 hrs following the end of the session. These control animals had enhanced NK activity compared to animals at the same time as other animals were being exposed to the actual shock. Consequently, the cues associated with the stressor (i.e., odors, sounds) may have served to provoke these changes in NK. Additional experiments were undertaken to test this hypothesis.

In contrast to mice which were neither handled nor exposed to the shock environment (Home Cage Control), non-shocked mice exposed to the apparatus again exhibited an increase in NK activity 24 hrs after handling. In a separate experiment, control cage controls were compared to mice exposed to footshock or to one of 2 non-shocked conditions. That is, one group was placed in the no shock apparatus at the same time as other animals were receiving shock (No Shock With), while the other group was segregated and exposed to the apparatus in a separate room (No Shock Alone). Consistent with the earlier results, only those animals exposed to the environment in which animals were being shocked (No Shock With) exhibited an increase in splenic NK activity. These data suggest that the responses of animals to a stressful situation may serve as signals to other animals that a threat is present. It may be speculated that in anticipatory changes occurring which would prepare the organism to respond to the environmental challenge. Consequently, the enhanced noted in NK activity may be the byproduct of increased sympathetic activation, or may be an anticipatory response to the fear of possible physical insult. Although the nature of the cues remains to be determined, these data do indicate that the environmental context in which a stressor is applied may be an important determinant of the immunological consequences of stress exposure.


Our research program is designed to systematically investigate the relationship between stress and immune function. Recently, the occurrence of the suppression of the immune system by drugs was found to occur in the stress in which the stressor is administered.

In our initial study, male Lewis rats received 4, 8 or 16 signaled footshocks (5 sec., 1.6 mA. V, L. 4 min.) in each of 1, 3 or 5 24 hr sessions. A weak footshock response to Con A was progressively reduced for both whole-blood and spleen lymphocytes with increasing numbers of shock presentations. However, the immune-suppression attenuated across sessions for the spleen lymphocytes, whereas the blood lymphocytes remained suppressed. The spleen and whole-blood lymphocytes also differed in the rate of recovery from shock exposure. The lymphocytes remained depressed within 24 hours, whereas the blood lymphocytes took between 48 and 96 hours to recover.

In a separate study designed to compare different environmental stressors and different physiological stress responses, 16 footshocks during a single 64-minute period produced a reduced responsiveness of splenocytes to PHA and Con A an increase in corticosterone levels. Stress responses to Con A and corticosterone were also increased in the control group of unshocked mice. These results indicate that the environmental context in which a stressor is applied may be an important determinant of the immunological consequences of stress exposure.

438.7 A VIRUS ACTIVATED STRESS RESPONSE IN MICE: CEREBRAL BIORHYTHM, PLATELET, CYTOTOXIC, AND IMMUNE RESPONSE PROLIFERATION, B.J. Dunn, M.E. Freytag, J.M. Gaskin and N.J. Hi, Departm ent of Pharmacology, College of Medicine, and Department of Infectious Disease, College Veterinary Medicine, University of Florida, Gainesville, FL 32610 and Department of Biochemistry, George Washington University School of Medicine, Washington, DC 20037.

We have investigated the potential use of a virus (Newcastle disease virus, NDV) as a stressor in mice. NDV, a chicken virus, is not infective in cells and provokes an immune response. NDV elevates body temperature about 2°C, maximal at about 12 hours. In a recent preliminary report, NDV (750 HA units IP) increased concentrations of plasma corticosterone (CS) and increased IL-1 levels in the spleen and peritoneal cavity. Furthermore, NDV also produced a profound decrease (60-90%) in the immunological response to the signal for shock inhibition. In a separate study, mice were exposed to unannounced 120 db sound for 1, 3 or 12 days did not affect immune response, but that the time between antigen administration and plaque formation was determined. A marked reduction (70%) of plaque formation was noted in mice that had been stressed 72 hr after antigen administration, while no effect was evident in mice that were exposed to the stressor 0.24 or 95 hours after antigen treatment. This effect was not related to the time between stress exposure and spleens being taken, since a reduction in plaque formation was also evident when the number of PFC's was determined 120 hr after antigen administration. Thus, the time between antigenic and exogenous stressor was fundamental in determining the immunological response. Additionally, the magnitude of the PFC suppression was directly related to the number of shocks mice received. Neither 10 nor 30 shocks affected plaque formation, while a marked reduction (30%) was evident following 60 shocks. Contrary to the PFC reduction in virtually all animals following 360 shocks, the effects of the 60 shock treatment appeared to be of an all-or-none nature, in that profound suppression of plaque formation was only evident in about half the mice.

In a subsequent experiment, uncontrollable and controllable shock were found to be equally effective in suppressing plaque formation. In contrast, the same stressor parameters were previously shown to influence central noradrenergic concentrations. Thus, the occurrence of the suppression of PFC's was not related to the organism's ability to contend with the nociceptive properties of shock. It has been hypothesized that stressful events may profoundly influence the immune response, but that the time between antigen administration and subsequent exposure to a stressor is critical in determining whether such an effect will be evident.

438.8 STRESSOR EFFECTS ON THE PLAQUE FORMING CELL RESPONSE TO SHEEP RED BLOOD CELLS, J. Shadley* J. A. Kates, R. H. Richter*, M. Richter*,and J. L. Hall, Psychology Department, Carleal University, Ontario, Canada KIS 5B6 and Dept. of Microbiology and Immunology, Dept. of Pathology, Faculty of Health Science, University of Ottawa, Ottawa, Ont. Canada, K1H 8M5.

A series of experiments assessed the effects of stressors on the plaque forming cell (PFC) response in mice. The effects of various intervals (0, 24, 72 or 95 hours) following administration of a submaximal dose of antigen (sheep red blood cells, 10⁶ cells/ml ip CD-1 mice were exposed to 360 shocks of 2 sec duration (150 uA). Spleens were taken 96 hr following antigen administration and plaque formation was determined. A marked reduction (70%) of plaque formation was noted in mice that had been stressed 72 hr after antigen administration, while no effect was evident in mice that were exposed to the stressor 0.24 or 95 hours after antigen treatment. This effect was not related to the time between stress exposure and spleens being taken, since a reduction in plaque formation was also evident when the number of PFC's was determined 120 hr after antigen administration. Thus, the time between antigenic and exogenous stressor was fundamental in determining the immunological response. Additionally, the magnitude of the PFC suppression was directly related to the number of shocks mice received. Neither 10 nor 30 shocks affected plaque formation, while a marked reduction (30%) was evident following 60 shocks. Contrary to the PFC reduction in virtually all animals following 360 shocks, the effects of the 60 shock treatment appeared to be of an all-or-none nature, in that profound suppression of plaque formation was only evident in about half the mice.

In a subsequent experiment, uncontrollable and controllable shock were found to be equally effective in suppressing plaque formation. In contrast, the same stressor parameters were previously shown to influence central noradrenergic concentrations. Thus, the occurrence of the suppression of PFC's was not related to the organism's ability to contend with the nociceptive properties of shock. It has been hypothesized that stressful events may profoundly influence the immune response, but that the time between antigen administration and subsequent exposure to a stressor is critical in determining whether such an effect will be evident.

domestic settings and their stressors. While certain behavioral responses to stressors are known to modulate a variety of pathophysiological changes, little is known about the influence of conspecific aggression on the immune system.

In the present study, aggressive responses were observed in all 6 mice (3lpr/lpr and 3Balb/cByJ), housed individually for 6 or 10 wks, were paired daily for 1 min on the same side of a 17 x 9 x 8 cage, which was divided in half by a wire mesh barrier. During 14 test sessions, behavioral interactions (e.g., dominance) were noted. Control groups included: mice paired across the barrier from each other (2lpr/lpr and 2Balb/cByJ) and "winners" mice paired across the barrier from a fighting pair. After 14 d the mice were sacrificed, and spleen and lymph nodes harvested. In vitro proliferative responses to lymphocyte cytotoxicity of T or B cell selective mitogens were examined and the activity of spleen natural killer (NK) cells was evaluated.

lpr/lpr mice showed more aggressive behavior than Balb/c mice. Strain differences for several behaviors were noted in dominant / submissive pairs. For example, chasing and biting the submissive mouse was more frequent in dominant mice.

Our results indicate that genetic factors and behavioral responses of dominant / submissive mice interact to modulate the impact of a natural stressor on the immune system.

438.10 AUTOMMUNE DISEASE MODIFIES CYCLOPHOSPHAMIDE INDUCED TASTE AVERSION IN MRL MICE. L. J. Grota, R. Ader*, and N. Cohen*. Dept. of Psychiatry and Immunology, University of Rochester Medical Center, Rochester, NY 14642 USA.

taste aversions in MRL mice. We have previously demonstrated that BALB/c mice have poorer taste aversion than +/+ mice as the dose and number of exposures increase.

We deprived lpr and +/+ mice were randomly assigned to treatment groups at 19-20 weeks of age. In the morning, a drinking bottle containing chocolate milk (200 ml, 2% homogenized whole milk) and 0, .1, .4, or 8 mg of cyclophosphamide per ml was placed on the cage for 1 hr. Consumption of the mixture was estimated by weighing the bottle before and after the drinking period. Supplemental water was made available in the afternoon. At weekly intervals, the mice were palpated for lymphoproliferation, eye bleed for serum ant-DNA antibody titers, and weighed.

In 2 replications, there was an inverse relationship between the amount consumed and the concentration of cyclophosphamide in the drinking solution. After 1 week of exposure, there were no differences between lpr and +/+ males irrespective of concentration. After 2 or more weeks of exposure, more cyclophosphamide than +/+ animals at low concentrations of cyclophosphamide (L 2 & 4 mg/ml). This pattern of consumption occurred earlier in males than females.

The dose of cyclophosphamide ingested was positively correlated with the concentration of cyclophosphamide in the drinking solution up to a maximum at .4 mg/ml. Lymphoproliferation and anti-DNA antibody titer were not altered in animals exposed to chocolate milk alone. In animals exposed to cyclophosphamide alone, lymphoproliferation and anti-DNA antibody titers were reduced as a function of both, concentration of cyclophosphamide and weeks of exposure.

In contrast to aggressive controls, MRL mice with autoimmune disease will voluntarily consume chocolate milk containing cyclophosphamide in an amount sufficient to modify their immunity but not to produce dysphoria. These results implicate behavior in the modulation of immune state.

This research was supported by NICHD and RJR-Nabisco, Inc.
SEROTONERGIC AND CHOLINERGIC INFLUENCE ON POSTERIOR CIRCUITRY OF THE MEDULLA OBLONGATA

The serotonergic influence on the medulla oblongata is mediated through a central nervous system mechanism. The serotonergic system consists of neurons located in the brainstem that project to various areas of the medulla oblongata, including the respiratory centers. These neurons are involved in the regulation of respiration, sleep, and other autonomic functions.

Cholinergic influence on the medulla oblongata is mediated through the central nervous system and is involved in the regulation of respiration, sleep, and other autonomic functions. The cholinergic system consists of neurons located in the brainstem that project to various areas of the medulla oblongata, including the respiratory centers. These neurons are involved in the regulation of respiration, sleep, and other autonomic functions.

IMMUNOCYTOCHEMICAL LOCALIZATION OF CARBONIC ANHYDRASE IN MEDIULLARY REGIONS OF RESPIRATORY CIRCUITRY

Carbonic anhydrase (CA) plays a crucial role in the regulation of respiration and other autonomic functions. It is involved in the conversion of carbon dioxide to bicarbonate, which facilitates the transport of carbon dioxide across the alveolar membrane. The localization of CA in the medullary regions of the respiratory circuitry is important for understanding the mechanisms of respiration and other autonomic functions.

CARBONIC ANHYDRASE ACTS THROUGH A CENTRAL NERVOUS SYSTEM STEROID RECEPTOR MECHANISM TO STIMULATE RESPIRATION

Modeling the effect of carbonic anhydrase on respiration requires understanding its localization in the medullary regions of the respiratory circuitry. The localization of CA in the medullary regions of the respiratory circuitry is important for understanding the mechanisms of respiration and other autonomic functions.

GABAERGIC REGULATION OF RECURRENT LARYNGEAL AND PHRENIC NERVE ACTIVITY IN THE CAT

The role of GABA in the regulation of recurrent laryngeal and phrenic nerve activity in the cat is important for understanding the mechanisms of respiration and other autonomic functions. The localization of GABA in the medullary regions of the respiratory circuitry is important for understanding the mechanisms of respiration and other autonomic functions.
POSTNATAL MATURATION OF ELECTROPHYSIOLOGIC PROPERTIES OF NEURONS IN THE VENTRAL REGION OF THE NUCLEUS TRACTUS SOLITARIUS IN THE RAT. G.G. Hagg and R.A. Getting. Dept. of Physiology and Biophysics, Univ. of Iowa, Iowa City, IA 52242.

Previous studies have shown that biochemical and morphologic changes occur with postnatal development in the brainstem of the rat. However, little is known about the electrophysiologic properties of neurons in the brainstem, particularly those involved with respiration. Since the inherent electrophysiologic properties of individual cells in a network can markedly influence the final output of that network, we examined postnatal maturation in these properties of premotor neurons in the ventral region of the Nucleus Tractus Solitarius (v-NTS).

1. Type A cells had a much higher rate of firing (10-20 spikes/sec) at rest, excitation (DE) which was manifested as a prolonged delay (up to 700 ms) to a test stimulus. These cells responded very vigorously to CO₂. Type B cells had a much lower rate of firing at rest (1-2 spikes/sec) and marked spike frequency adaptation (SFA) with depolarization. Most type A cells had shown little activity. These results suggest that the rhythmic discharge patterns of v-NTS neurons in the rat are not determined by a single synaptic input from a fixed source. The rhythmic discharge patterns are controlled by a network of interconnected neurons. The present study was supported by the National Institute of General Medical Sciences (GM-13169) and the American Lung Association and HRSF (United Way).


The discharge characteristics of phrenic motoneurons are strongly influenced by synaptic connections that are established after birth. The surface area available for receipt of these synaptic connections is determined by the rate of growth of the cell membrane. We set out to determine the developmental changes in phrenic motoneurons during the first postnatal months when these neural elements controlling the diaphragm play such a critical role in sustaining ventilation of the newborn.

Kittens in three age groups (1-2, 4-5 and 7-8 weeks) were selected for study. The CO₂ root of the phrenic nerve was exposed and sectioned after aseptic surgery under halothane anesthesia. The central end of this root was soaked in a 40% HRP solution for 30-60 minutes. After a 48-hour survival time, the animal was deeply anesthetized and transcardially perfused. The cervical spinal cord was removed and cut into 400-450 μm coronal sections and reacted with TMB. The outline of cell body and proximal dendrites of heavily labeled motoneurons were reconstructed using a 10X oil objective. The major and minor axes, perimeter (p) and cross-sectional area (A) were measured from these drawings using a digitizing tablet. The number of primary dendrites was recorded. In addition to the morphologic parameters measured, average diameter, form factor, cell surface area and volume were calculated.

Fifty cells from each animal (two kittens per age group) were analyzed. The mean values (standard error) for the different age groups are summarized below.

<table>
<thead>
<tr>
<th>Age group (weeks)</th>
<th>A (μm²)</th>
<th>d (μm)</th>
<th>E (μm²)</th>
<th>D (μm²)</th>
<th>p (μm)</th>
<th>A (μm²)</th>
<th>V (μm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>28.2</td>
<td>2.7</td>
<td>67.2</td>
<td>50.7</td>
<td>2.0</td>
<td>78.0</td>
<td>431.5</td>
</tr>
<tr>
<td>4-5</td>
<td>34.1</td>
<td>3.4</td>
<td>89.1</td>
<td>64.6</td>
<td>2.5</td>
<td>101.3</td>
<td>504.3</td>
</tr>
<tr>
<td>7-8</td>
<td>39.1</td>
<td>3.9</td>
<td>124.2</td>
<td>80.6</td>
<td>3.0</td>
<td>142.5</td>
<td>635.8</td>
</tr>
</tbody>
</table>

With the exception of the number of primary dendrites between all age groups and the form factor between the first and second age group, all other values differed significantly (p<0.01) between age groups. Over the age ranges studied, the surface area has almost doubled while the number of primary dendrites has remained unchanged.

Supported by grants from NIH (HL26375) and N022762, American Lung Association and HRSF (United Way).
TRIPHASIC PATTERN OF MEMBRANE POTENTIAL IN RESPIRATORY NEURONS OF NEONATE PIGLETS. R. E. Lawson*, D. W. Richter*, B. L. Brown, and D. Ballantyne*, Dept. of Pediatrics, Univ. of North Carolina at Chapel Hill, and 1. Physiologisches Institut der Universitaet Heidelberg, FRG.

The objective of the present study was to determine the pattern of phasic activity of individual respiratory related neurons and to determine whether synaptic inputs affect these patterns in newborns. Respiratory neurons were recorded in 12 piglets, 2-13 days of age. The piglets were anesthetized with pentobarbitol, intubated, paralyzed, ventilated, and mounted in a rigid frame. The dorsal medulla was cut bilaterally and a glass microelectrode was placed into the C2 spinal cord to test for antidromic activation of bulbospinal neurons (5-20V, 0.05ms pulses). Intracellular recordings of neurons were obtained using glass microelectrodes 20-60 megOhms resistance containing NMDG or 2M Kacetate. Reversal of cyclic membrane potential patterns (and E or negative current injection was taken as evidence of inhibitory post-synaptic (ips) activity. Respiratory neurons were identified in two caudal medullary regions with stereotactic coordinates similar to the dorsal and ventral respiratory groups (DRG and VRG) of the adult cat. Neurons were classified according to the respiratory phase in which dephosphorylation occurred. In one animal, 2 bulbospinal DRG inspiratory units were recorded both of which received excitatory post-synaptic potentials following ipsilateral vagus stimulation. Respiratory neurons were recorded in the lateral medulla (eg the VRG) of the remaining 11 piglets. Thirteen inspiratory neurons (7 bulbospinal, 13 postinspiratory neurons (6 vaginal), and 27 respiratory neurons (13 bulbospinal) were recorded (initial membrane potential >35mV) at least for enough time to allow respiratory phase verification. In contrast to the usual two phase pattern of phasic activity (eg ramp inspiration and expiratory silence), the neuronal discharge consisted of hyperpolarization, depolarization, and apparent disinhibition. For each of the three cell types the respiratory phase contained two distinct periods of membrane potential reversal of ipsps during the postinspiratory or later expiratory phases was demonstrated in inspiratory neurons (17 cases) similar to that seen in the adult cat. A biphasic pattern of membrane activity in the respiratory neurons was similar that of the adult cat (Brown 1985). These data, from newborns, support the three phase theory of respiratory cycle control demonstrate that inhibitory synaptic inputs dominate central respiratory patterns. Supported from NIH HL34619 and WHO600475, Alexander von Humboldt Stiftung, and DFG.


Cross-correlation analysis of simultaneously recorded neurons located in the lateral side of the medulla revealed best rates for at least two synaptic processes in the development and termination of each phase of breathing (1,2). Respiratory neurons were classified according to locations of constituent cells (rows) and phase of breathing (1,2). Recordings made following midsagittal section of the lateral medulla (eg the VRG) of the remaining 11 piglets. Thirteen inspiratory neurons (7 bulbospinal, 13 postinspiratory neurons (6 vaginal), and 27 respiratory neurons (13 bulbospinal) were recorded (initial membrane potential >35mV) at least for enough time to allow respiratory phase verification. In contrast to the usual two phase pattern of phasic activity (eg ramp inspiration and expiratory silence), the neuronal discharge consisted of hyperpolarization, depolarization, and apparent disinhibition. For each of the three cell types the respiratory phase contained two distinct periods of membrane potential reversal of ipsps during the postinspiratory or later expiratory phases was demonstrated in inspiratory neurons (17 cases) similar to that seen in the adult cat. A biphasic pattern of membrane activity in the respiratory neurons was similar that of the adult cat (Brown 1985). These data, from newborns, support the three phase theory of respiratory cycle control demonstrate that inhibitory synaptic inputs dominate central respiratory patterns. Supported from NIH HL34619 and WHO600475, Alexander von Humboldt Stiftung, and DFG.


The parabrachial nuclei (PB), comprising a "pneumotaxic center," is recognized as necessary for maintaining normal respiratory rhythm. Electrical stimulation of PB elicits various respiratory effects including periodic breathing, but no clear picture of the functional organization of the PB has emerged. Recent findings on the cytoarchitecture and connections of PB (S. Res Biv 1984; 7:229), we have re-examined the functional organization of PB subnuclei and their relation to the respiratory control. The respiratory airflow and blood pressure were monitored in a chloralosed, anesthetized, spontaneously breathing rat during electrical stimulation (15 sec trains of 50 Hz, 0.25 msec pulses) of PB subnuclei using glass microelectrodes filled with 2 M KCl and pontamine sky blue to mark each electrode track. Respiratory responses, threshold currents, and electrode coordinates were recorded for each stimulation site every 100 μm in tracks spaced 200 μm apart. Following each experiment, the brain was fixed in 10% formalin, sectioned at 50 μm and stained with thionin. Maps of PB subnuclei and electrode tracks were made by drawing the sections using a projection microscope and recording the electrode coordinates. The effective stimulation sites were plotted on them. Changes in tidal volume and respiratory rate were obtained at stimulating PB parvocellular, parvocellular, and parvocellular nuclei of the thoracic-pneumotorubal. Hyperspinal was produced by stimulation of the external or the central lateral subnucleus. Hypocapnia or apnea was produced by stimulation of the external subnucleus. Additional respiratory responses were obtained by stimulating the external or the central lateral subnucleus, or the medial subnucleus. Some of these responses seemed to depend on the rostral-caudal level which was stimulated. These results demonstrate that opposite respiratory effects can be achieved by stimulating different subnuclei of the PB which have different projections. By combining microstimulation with anterograde neuronal tracing, it should be possible to identify specific cell groups and pathways that participate in discrete respiratory pattern control. N.R.H. is an ARA Research Training Fellow. N.H. is a DAAD/NATO Research Training Fellow.

Low intensity electrical stimulation of the rostral pontine respiratory group (PRG) produced altered inspiratory respiratory pattern; we studied the effects of PRG stimulation on the membrane potential (Vm) of expiratory-related (E) neurons of the ventral respiratory group (VRG). Experiments were performed on anesthetized, paralyzed, vagotomized and mechanically ventilated cats. Stimulation was made with a tungsten electrode (~2 MΩ) inserted into the VRG. Intracellular recordings (3 M KCl) were made from ipsilateral VRG E cells. Among 16 E cells with a Vm < -40 mV, 10 were bulbospinal, 3 laryngeal and 3 were not antidromically activated. Only 4 VRG E cells could be activated synthetically by single pulse stimulation of the PRG (latencies: 12-20 ms). Within the range of PRG stimulation used (30-50 μA, 100 Hz, 0.2 ms), effects on Vm were inconsistent and were delivered during early inspiration (I) and early E. Low intensity stimulation of PRG during early I, which depressed phrenic nerve activity, transiently depolarized E neurons without increased stimulus intensity associated with inspiratory phase termination. Vm exhibited a post-inspiratory shift characterized by a steep depolarization leading to E discharges. With stimulation during late I, Vm exhibited a post-inspiratory shift independent of stimulus intensity. PRG stimulation during post-inspiration, which abolished post-inspiratory activity of the phrenic nerve, increased the rate of depolarized E, E neurons, leading to earlier initiation of the E spike burst. PRG stimulation at low intensity during mid-E caused little or no change in Vm, but with increased intensity, induced a transient hyperpolarization. Stimulation during late E, terminating the E phase, was marked by a sharp hyperpolarization of Vm, suggesting an enhanced early inhibition. That the intensity-dependent effects occurred only during early I and early E suggest that PRG influences on respiratory pattern are mediated or modulated by early I and post-inspiratory cells. Supported by NIH Grant HL 37941.


The spectral composition of inspiratory (I) nerve activity (phrenic (PHR) and recurrent laryngeal (RL)) and specifically the strength of high-frequency oscillations (HFOs, range 50–100 Hz) and medium-frequency oscillations (MFOs, range 20–50 Hz) is known to vary during I (Richardson & Mitchell, Brain Res., 253, 317–336, 1982). It has also been observed that early-I PHR activity and it has been suggested that their occurrence later in I is due to activity of late recruited phrenic motoneurons (H. Wehr, Soc. Neurosci. Abstr. 13, 305, 1986). Since medullary (DRG and VRG) I neurons and also cervical I neurons show HFO coherence to the PHR HFO, we studied the behavior of these cycles at different stages of I is of interest. We therefore performed spectral analysis on spike trains of DRG and VRG I neurons, as well as on activity of PHR and in some cases RL nerves, all obtained from decerebrate cats that were paralyzed and ventilated with a cycle-triggered pump. To study the changes during I, the activities were fed to different parts of I (e.g., halves), including the start of I (initial stage lasting 20–50 ms or often shows a PHR burst). Our data showed that: (1) PHR and RL I activity can exhibit HFO or MFO or both at all times during I; (2) there is no MFO in PHR, MFOs, which are not coherent between PHR and RL, tend to increase in amplitude throughout I phase. The MFO grows or stays the same for up to two thirds of I and becomes weaker or even vanishes in late I. The PHR-RL coherence of the HFO changes in a similar way. (2) DRG and cervical I neurons show no MFO at any stage of I. Late I, no MFO; (3) VRG neurons have a transition from the HFO to a somewhat lower-frequency rhythm which, unlike the HFO, can not be synchronized to any high-frequency components of PHR and is not coherent to PHR. The MFO of medullary and cervical neurons changes during I in a way similar to the PHR MFO, and the phase coherence they maintains. When the PHR HFO vanishes in late I, the unit HFO and the coherence do not have MFOs. These observations indicate that MFOs are specific to different MN pools and in PHR are unlikely to originate from pre-motor networks in the manner the HFO does. MFOs might arise from subsets of MNs having rhythms in the MFO range, and their changes during I could then reflect changes in the distribution of the properties of the active MNs. In conclusion, the spectral properties of the respiratory system is likely to arise in a common pattern generator. The changes of HFO during I may provide information on the time course of interactions of I neuron populations. (Supported by N.I.H. Grant HL-27500.)


Cross correlation analyses in mammals have demonstrated that locomotor and respiratory cycles are synchronized to some degree during locomotion, although the mechanisms for the coupling remain unclear. We examined the importance of telencephalic structures to the synchronization of locomotor and respiratory cycles in birds, as well as the effects of changes in the mode of locomotion on this synchronization. Decerebrate Canada geese were induced to walk (3–5 mm) or wing flap (up to 30 sec) by electrical stimulation (25–100 μA, 60 Hz) of the dorsomedial paracervical reticular formation (PRF) or the ventromedial gigantocellular reticular formation (Rgc).

Minute ventilation (Ve), the product of ventilation frequency (f) and tidal volume (Vt) was monitored via pneumotachography. Stepping and wing beat motor patterns were recorded with electromyographic techniques. Breathing and locomotor frequencies were measured over 1–10 min intervals and the degree of synchronization was recorded as a function of elapsed time into exercise.

During walking, ratios of 2 or 4 steps per breath were most commonly found and this synchronization took 40–60 sec after the start of walking. When the treadmill speed was altered, the breathing pattern readjusted to match the new stepping frequency within 20–40 sec. In intact animals, respiratory and wing beat frequency became synchronized almost immediately which may reflect an obligatory respiratory-motor coupling of the two muscle systems. The onset of flight movements was associated with a dramatic change in breathing pattern whereby a 5–10 fold decrease in Ve was associated with a 10–25 fold increase in f resulting in the maintenance of a relatively constant level of alveolar ventilation.

These data suggest that the mechanisms responsible for the synchronization between respiratory and locomotor cycles do not require forebrain structures and that these mechanisms differ for cervical and diencephalic activation. Supported by NSERC of Canada.

439.16 POST-HYPOXIC FACILITATION AND INHIBITION OF RESPIRATION IN GLOMOCENTRONIC CATS. R.A. Gallan and D.B. Millhorn, Department of Physiology, University of North Carolina, Chapel Hill, North Carolina.

The respiratory response following hypoxia was studied in peripheral chemodenervated cats with intact brains and following bilateral decerebration. Two cycles are synchronized to some degree during locomotion, although the mechanisms for the coupling remain unclear. We examined the importance of telencephalic structures to the synchronization of locomotor and respiratory cycles in birds, as well as the effects of changes in the mode of locomotion on this synchronization. Decerebrate Canada geese were induced to walk (3–5 mm) or wing flap (up to 30 sec) by electrical stimulation (25–100 μA, 60 Hz) of the dorsomedial paracervical reticular formation (PRF) or the ventromedial gigantocellular reticular formation (Rgc).

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These data suggest that the mechanisms responsible for the synchronization between respiratory and locomotor cycles do not require forebrain structures and that these mechanisms differ for cervical and diencephalic activation. Supported by NSERC of Canada.
439.17 Yawning: No Effect of CO₂, O₂, and Exercise.  B. R. Provine, B. C. Tate, and L. L. Getmaner, Dept. Psychol., Univ. of N.C. Baltimore County, Catonsville, MD 21228

Using human and age-matched subjects, the present study tested the commonly cited but previously untested hypothesis that yawning is facilitated by higher than normal levels of O₂ or lower than normal levels of O₂ in the blood by comparing the effect on yawning of breathing 100% CO₂ and gas mixtures with higher than normal levels of CO₂ (31 or 54%) with compressed air, the control condition. If yawning is a response to heightened blood CO₂, the CO₂ mixtures should increase yawning rate and frequency. If low blood CO₂ produced yawning, breathing 100% O₂ should inhibit yawning. The CO₂/O₂ hypothesis was rejected because breathing neither pure CO₂ nor gases high in O₂ had a significant effect on yawning although both increased breathing rate. A second study found that exercise sufficient to double breathing rate had no effect on yawning. The two studies suggest that yawning does not serve a primary respiratory function and that yawning and breathing are triggered by different internal states and are controlled by separate mechanisms.

Further evidence that yawning is not primarily a respiratory act comes from an analysis of the routes of inhalation and exhalation during yawning and the relation between yaw duration and inter-yawn interval (also see Provine, R. R., Ethology, 72, 109-122). Inhalation and exhalation occur primarily through the mouth and this basic pattern is highly inflexible. It is very difficult, if not impossible, for most people to perform a satisfying yawn with their mouth taped shut even though they are free to respire through their nose and move their jaw to a limited extent within their closed mouth. Yawning does not have the degree of behavioral freedom characteristic of normal breathing. However, oral inhalation by itself is insufficient to produce a satisfactory yawn if the jaw is not free to move. Subjects attempting to yawn with clamped teeth often report the unpleasant sensation of being stuck mid-yawn and being unable to perform a satisfying yawn although they could inhale and exhale through their teeth. Thus, the yawn is not simply a deep breath. The finding that perpetrators of short yawns do not compensate by yawning more frequently and vice-versa is also inconsistent with the yawn as a respiratory event.

439.18 EFFECT OF HIGH PRESSURE ON SPONTANEOUSLY ACTIVE RESPIRATORY DRIVE IN ISOLATED MEDULLA OF NEWBORN RATS.  A. Parasig* and Y. Grossman*  Unit of Physiology, Faculty of Health Sciences, Coresh Center for Medical Sciences, Ben Gurion University of the Negev, Beer Sheva 84105, Israel.  (SPON: K.R. Courtenay)

High pressure, which induces hypercapnia and convulsions in intact animals (High Pressure Nervous Syndrome), slows respiratory rate and increases respiratory duty cycle. In newborn rats, these effects are associated with disrpes. The present experiments were designed to distinguish the central effects of pressure from the direct effects of compressed gas on lung mechanics. The medulla and spinal cord of newborn rats were isolated, placed in a pressure chamber and constantly superfused with oxygenated (95% O₂ 5% CO₂) bicarbonate-buffered (pH 7.3) Ringer solution at 27°C. Spontaneous respiratory activity indicative of "respiratory drive" was recorded extracellularly from the cut C-4 and C-5 ventral roots, while give rise to the phrenic nerve. Changes in rhythmic activity were induced by extracellular stimulation of the fifth cranial nerve. After control measurements at 1 atm, the pressure was raised to 100 atm with compressed helium. Exposure to high pressure caused a reduction in the rate of spontaneous activity by 50%-60% at the amplitude of each spontaneous burst of activity by 10-20%, and increased its time integral by 50%, brief (10-20 sec) stimulation of the fifth nerve at 10 Hz and 1 atm transiently (30-40 sec) increased the frequency of respiratory drive and decreased the durations of individual bursts of activity. High pressure prolonged this period of increased frequency to minutes, without significantly affecting the durations of individual activity bursts. Stimulation of the fifth nerve at 1-5 Hz for 1 min had no effect at 1 atm. However, when delivered under high pressure, this stimulation induced an entirely different pattern of spontaneous activity in the ventral roots. These results demonstrate for the first time a direct pressure response of the neuronal centers which control respiration. Although spontaneous activity is suppressed under pressure at steady state, the system is nevertheless rendered hyporesponsible to repetitive sensory stimulation, and net respiratory drive may therefore rise to above the control level.

PAIN MODULATION V

440.1 COCAINE: MECHANISMS OF CNS ANALGESIC ACTION IN THE RAT.  Yu Lii*, T.J. Morrow & R.J. Casey, Dept. of Neurology & Physiology, Univ. of Michigan and Neurology Research Laboratories, VA Hospital, Ann Arbor, MI 48105

Recently, we showed in the rat that systemically administered cocaine (HCl, 25 mg/kg, i.p.) acts as a weak opiate, non-sedative, centrally acting analgesic as determined by both tail-flick (n=30) and formalin (n=24) tests. Reduction of pain behavior in the formalin test was immediate (within 5 minutes), complete (pain score drop from 1.5-2.0 to 0-0.5-2) and sustained (lasting more than 1 hour). Accordingly, we sought to determine the mechanism(s) involved in cocaine analgesia. Using the formalin test to access changes in pain behavior, we attempted to block the analgesic effects of cocaine using a known opiate (naloxone, 1 mg/kg, i.p.) and dopamine (chlorpromazine, 3 mg/kg, i.p.) antagonists. We also compared the analgesic effect of cocaine with those produced by morphine (6 mg/kg, i.p.) and salbutamol (2.5 mg/kg, i.p.). We found that cocaine HCl, given intraperitoneally, produced a profound behavioral analgesia in a dose-dependent fashion, such that 20 or 25 mg/kg caused complete loss of pain responsiveness, while 15 and 10 mg/kg doses showed progressively weaker and shorter duration effects. Five mg/kg doses of cocaine had no analgesic action. Cocaine also failed to produce analgesia when administered by either intramuscular or subcutaneous injection. Naloxone, which reversed the effect of morphine, had no effect on the pain behavior of controls or cocaine treated animals (n=10). In contrast, chlorpromazine partially (n=9) or completely (n=3) blocked the analgesia produced by cocaine when given concurrent with (n=6) or 30 minutes prior (n=6) to cocaine. In rats showing partial attenuation of cocaine analgesia, the onset was more gradual and the time course significant in most cases. Chlorpromazine had no effect on the pain behavior of control animals or on the analgesia produced by morphine. In conclusion, these data clearly show: (1) that in the rat, systemically administered cocaine HCl has a profound and dose-dependent central analgesic action and (2) that this analgesia is mediated by non-opiate mechanisms.

Supported in part by grants from NIH, AFSR and the Veterans Administration.

440.2 COCAINE: NEUROPHYSIOLOGICAL EFFECTS ON BULBORETICULAR PROJECTIONS IN THE RAT.  C. R. Belczynski*, A. Pertovaara, T. J. Morrow and R. J. Casey, Dept. of Neurology & Physiology, Univ. of Michigan and Neurology Research Laboratories, VA Medical Center, Ann Arbor, MI 48105

We have shown (Lin et al, this meeting) that in the rat, cocaine-HCl (25 mg/kg, i.P.) is a rapid onset (<10 min.), centrally acting analgesic that is neither sedative nor opiate-mediated. We are studying possible neurochemical basis for this effect through extracellular recording of changes in the spontaneous and evoked activity of antidromically identified bulboreticular neurons in rats anesthetized with chloral hydrate.

Twenty-three neurons were recorded from the medial medullary raphe, gigantocellular, or paragigantocellular nuclei. Of 20 spinally projecting neurons, 8 showed a prolonged increase, 2 a decrease, and 10 no change in the level of spontaneous activity within 10 minutes of cocaine injection. Thirteen neurons were responsive to noxious somatic stimulation. Of these, 10 (9) reduced their responsiveness after cocaine, with the remaining showing an increase (2) or no change (2). The increased responsiveness of one of these neurons was abolished within 3 minutes of the administration of chlorpromazine (3 mg/kg, i.p.). The three remaining bulboreticular neurons projected centrally through the medial forebrain bundle and showed immediate (5 min.) cocaine-induced increases in spontaneous activity accompanied by a concurrent reduction in responsiveness to noxious stimulation.

These findings provide direct evidence that cocaine, in doses analgesic for rats, affects both spontaneous and nociceptively-evoked activity of caudally and rostrally projecting bulboreticular neurons over a time course paralleling its central analgesic effects. The observation that somatic responsiveness was reduced while spontaneous activity was unchanged or increased in most cells suggests a remote or presynaptic inhibition.

Supported by NIH, NSF and Veterans Administration.
440.3 MUTUAL POTENTIATION OF ANTIINOCICEPTIVE EFFECTS OF COCAINE AND MORPHINE. S. Lei* and G. L. Wilcox. Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455.

It is well known that either noradrenergic or opioid agonists alone inhibit spinal nociceptive transmission to ascending systems. Further studies in this and other laboratories have indicated that these two classes of spinally active antiinociceptive agents not only possess independent antiinociceptive effects, but also show mutual potentiation. Most studies of opioid-noradrenergic systems have involved morphine together with norepinephrine or clonidine. The present study sought to examine the interaction between morphine and cocaine, the latter agent having abuse liability, indirect noradrenergic agonist effects and local anesthetic effects. Naloxone and phentolamine were used to identify the receptor populations involved, and substance P (SP) was injected intrathecally (i.t.) to elicit scratching behavior.

Male mice (unanesthetized, 17-23 g) were given intrathecal (i.t.) injections by percutaneous lumbar puncture. This procedure involves directing a 30-gauge needle into an intervertebral space at approximately the level of the 5th or 6th lumbar vertebrae. Intrathecal application of SP in the mouse results in a caudally directed biting and scratching behavior possibly indicating nociception.

SP-elicited behavior was inhibited significantly by cocaine in a dose dependent manner in a dose range from 0.1 to 5 μg. The inhibitory effect of cocain (4μg, i.t.) on SP-elicited behavior was antagonized by co-administration of 0.1μg phentolamine.

Morphine (0.1μg) shifts the cocaine dose response curve about 10 fold to the left. Cocaine (0.5μg and 2.5μg) shifted the morphine dose response curve about 5 fold and 10 fold, respectively, to the left. The combined effect of morphine (0.5 μg, i.t.) and cocaine (0.5 μg, i.t.) was not blocked by 1 mg/kg naloxone. (c.b) but was significantly blocked by 1 mg phentolamine (i.t.).

Subcutaneous injection of morphine (2 μg/kg, a dose having no significant effect on SP-elicited behavior) increased the effect of cocaine (0.25 μg, i.t.), but complete dose response curves have not been completed. The combined effect was reduced by subcutaneous injection of naloxone (1 mg/kg).

These results suggest that cocaine exerts spinal antiinociceptive effects through a-norenergic receptors similar to direct-acting noradrenergic agonists, potentiates the spinal antiinociceptive effects of morphine.

(Supported by USHS grants DA-01933, DA-04274 and DA-04090).

440.4 MORPHINE INCREASES SUBSTANCE P IN THE DORSAL HORN DURING A NOCICEPTIVE STIMULUS. K. E. McGregor, B.D. Goldstein, M.I. Kity. Department of Pharmacology and Technology and Anatomy, Medical College of Georgia, Augusta, GA 30911.

The undecapeptide Substance P (SP) found in spinal cord, dorsal horn and peripheral nerves elicits scratching behavior. Substance P has been shown to increase the levels of SP and substance P-like immunoreactivity through quantitative immunohistochemistry. Morphine exerts an antiinociceptive effect which might be due to the ability of SP to attach to opiate receptors found on primary afferent terminals. In order to study the effect ofmorphine on SP levels in the dorsal horn, female Wistar rats were pretreated with subcutaneous injections of morphine sulfate (NS) 10 mg/kg or saline 1.0 ml/kg. Rats were anesthetized with urethane and NS formalin or saline (0.4 ml) was injected into the plantar aspect of the right hind paw. Perfusion with 4 paraformaldehyde and removal of the lumbar enlargement of the spinal cord followed after 1 hour. SP was quantitated using immunohistochemical staining and manual photocopying. Animals were grouped according to the pretreatment and the substance injected into the hindpaw. The four groups were saline-saline (pretreatment-hindpaw), NS-saline, saline-formalin, and NS-formalin. The results show that in formalin animals SP increased by 53% in the ipsilateral dorsal horn compared to saline-saline animals. This increase was somewhat less than the sum of these increases found in saline-formalin animals (34%) and NS-saline animals (36%) as compared to saline-saline animals. In summary, these results confirm that SP in the dorsal horn can be increased by a chemical nociceptive stimulus and show that pretreatment with morphine produces an acute increase in SP in the dorsal horn. These data also show that morphine can further increase SP in the dorsal horn during a nociceptive stimulus. Supported by NS18664.

440.5 SYSTEMIC ADMINISTRATION OF NALOXONE OR NALTREXONE PRODUCES ANALGESIA IN BALB/C MICE IN THE FORMALIN PAIN TEST. A.L. Vaccarino, R. Melzack*, and J.A. Tasker*. (SPON: M.G. Murphy), Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada.

Activation of opioid and a-adrenergic receptors inhibits adenylyl cyclase in cultured neuroblastoma-glioma hybrid cells, and this inhibition is blocked by pertussis toxin (J. Biol. Chem. 1981, 258:4870). Baclofen-induced inhibition of adenylyl cyclase in cultured granule cells also is blocked by pertussis toxin (J. Pharmacol. Exp. Ther. 1986, 239:548). In spinal cord gangeria cultures, pertussis toxin blocks depressant effects of morphine and noradrenaline on dorsal horn network responses (Brain Res. 1987, 400:185), while in cultured dorsal root gangeria cells, pertussis toxin blocks noradrenaline and GABAergic inhibition of calcium channels (Nature 1986, 319:670). Such observations suggest involvement of the inhibitory guanine nucleotide regulatory unit and possibly adenylyl cyclase in spinal actions of morphine, noradrenaline and baclofen. In the present study, we examined the effect of intrathecal (i.t.) pretreatment with pertussis toxin on antinociception produced by i.t. injection of morphine, noradrenaline and baclofen. Rats were implanted with chronic indwelling i.t. catheters and 2-3 days later percutaneous toxin (0.25-0.75 μg) or vehicle (phosphate buffer) injected intrathecally. Antinociception was tested 2-7 days later using the formalin test. After initial baseline flick latencies were determined, test drugs were injected and tail flick latencies measured at 15 min intervals for 60 min. Data was reassessed using ANOVA (all area under curve) and in the table is expressed as % control responses in vehicle-pretreated rats tested on the same day (n in brackets).

<table>
<thead>
<tr>
<th>Test agent</th>
<th>day 2</th>
<th>day 4</th>
<th>day 7</th>
<th>P.Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine 3.7-5 μg</td>
<td>46 ± 15(35)</td>
<td>35 ± 14(30)</td>
<td>10 ± 9(7)</td>
<td>0.5 μg</td>
</tr>
<tr>
<td>Baclofen 0.1-0.6 μg</td>
<td>47 ± 13(31)</td>
<td>39 ± 12(12)</td>
<td>28 ± 11(7)</td>
<td>0.25 μg</td>
</tr>
<tr>
<td>Noradrenaline 20 μg</td>
<td>21 ± 7(13)</td>
<td>10 ± 6(7)</td>
<td>7 ± 4(5)</td>
<td>0.5 μg</td>
</tr>
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<td>39 ± 11(6)</td>
<td>10 ± 10(9)</td>
<td>0.5 μg</td>
</tr>
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</tr>
<tr>
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<td>21 ± 7(13)</td>
<td>10 ± 6(7)</td>
<td>7 ± 4(5)</td>
<td>0.5 μg</td>
</tr>
</tbody>
</table>

The table indicates pertussis toxin inhibited antinociception produced by all three agents. These results suggest involvement of the inhibitory guanine nucleotide regulatory unit in the spinal antinociceptive action of morphine, noradrenaline and baclofen. However, the involvement of adenylyl cyclase remains to be determined. (Supported by NSERC Canada)
440.7 THE EFFECT OF MORPHINE ON PERIAQUEDUCTAL GRAY NEURONS MAINTAINED IN VITRO. S.A. Chandler* and M.M. Behbehani (SPON: D.L. Ross). Department of Physiology and Biophysics, U. Cincinnati College of Medicine, Cincinnati, OH 45267-0756.

In the past three decades it has been established that injection of morphine (MO) into the PAG produces strong and long lasting analgesia. Although the specificity of the action of morphine has been well established, the mechanism by which it produces analgesia is not clear. Experiments using electrical stimulation and injection of excitatory neurotransmitters such as glutamic acid and substance P into the PAG suggest that excitation of PAG neurons can consistently be demonstrated whereas lesions of PAG or injection of local anesthetics into this region seldom lead to modulation of pain perception. Since in most CNS sites, injection of MO produces a reduction in the firing rate and excitability of the neurones, it has been suggested that MO injected into the PAG produces analgesia through the process of disinhibition. To test this hypothesis, we have examined the effect of morphine on PAG slices maintained in normal physiological saline solution (PSS) and in calcium free solution instead of calcium containing solution (PSS-Co).

Adult male Sprague-Dawley rats were used. Animals were anesthetized with ether and then decapitated. The brain was removed and a three mm section surrounding the sagittal sinus was cut. This tissue was then sectioned into 450 micron slices. After incubation for one hour, one slice was placed in the recording chamber and was covered by a nylon mesh. The tissue was continuously perfused with oxygenated PSS at a rate of 3 ml/min. Extracellular recording was made from all regions of PAG using a glass electrode. Morphine was applied directly to the cell or through bath application. The specificity of the MO effect was tested by pretreatment with naloxone.

The effect of MO was examined in 233 cells in 85 animals. Only 40 (17%) of the cells were responsive to MO application. In this group, 24% were excited by morphine and the rest were inhibited. The inhibitory effect of morphine was dose dependent and could be reversed by naloxone. The excitatory effect of morphine was seen at a higher dose and was only partially reversed by naloxone. Both the inhibitory and the excitatory effects of morphine were observed even when the tissue was incubated in PSS-Co solution.

It is concluded that the major effect of morphine on the PAG neuron is inhibitory. The present study could be done while the synaptic transmission was blocked, indicating that MO produces its action through a postsynaptic mechanism. These studies suggest that the analgesic effect of morphine is likely to be due to the inhibition of inhibitory neurones. Therefore, it is possible that MO given directly onto the project outside the PAG rather than disinhibition of PAG interneurons. Supported by PHS Grant NS 20643.


The potentials elicited by stimulation of the isolated sciatic nerve in the tail of the anesthetized rat were recorded from electrodes placed over the surface of the somatosensory cortex. Recordings were made bilaterally and the latency of the evoked potential was observed contralaterally to the stimulation site. The potentials evoked by sciatic nerve stimulation were insensitive to neuromuscular blockade by d-tubocurarine (0.8 mg/kg sc), and tested on the tail flick for six consecutive tests. It is concluded that...

It is well established that, in rodents, intracerebroventricular (ICV) administration of morphine and related compounds results in powerful analgesia. Recently ICV morphine has been introduced as a form of pain relief in humans, especially for the relief of intractable cancer pain. However, the neurophysiological basis of such an action remains to be clarified. Convergent neurons are one of the prime candidates for processing nociceptive information at both spinal and trigeminal levels. The convergence of various types of information, both non-noxious and noxious origins onto these neurons predisposes this neuronal population to a global reception of sensory information which could be interpreted by the higher centers as basic somesthetic activity (BSA). We have proposed that the occurrence of a nociceptive event on an area of the body would perturb this BSA in two complementary ways: by activation of the corresponding segmental pool of neurons and by an inhibition mediated by Diffuse Noxious Inhibitory controls (DNC) which project supraspinally from the remaining extrasegmental neuronal population. The contrast response between the smaller excited and the large inhibited pool of neurons may well constitute a nociceptive signal information to the brain.

We present here evidence that morphine (10 μg) administered within the third ventricle in anesthetized rats completely blocks DNC acting on the BSA. DNC were triggered by the immersion of the tail in a 48°C waterbath, and BSA was simulated by responses of convergent neurons recorded at the lumbar level to innocuous mechanical stimulation but not to noxious thermal stimulation in the rat (In: Subacute Neurokinin A [sub-A] rat, morphine sulfate [MS] and noradrenaline [NA] inhibit release of SP in spinal cord (A. S. Advokat, Brain Res., 376: 115-118, 1986)). Antibody to substance P (AntiSP) is long-lasting (i.e. > 60 min) and was antagonized by systemic naloxone (0.4 mg/kg). We have used systemic naloxone for the antagonist of this action in the present study.

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Moreover, morphine sulfate (MS) and noradrenaline (NA) inhibit release of SP in spinal cord (Advokat et al., Brain Res., 376: 115-118, 1986). Antibody to substance P (AntiSP) is long-lasting (i.e. > 60 min) and was antagonized by systemic naloxone (0.4 mg/kg). We have used systemic naloxone for the antagonist of this action in the present study.

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Intracerebroventricularly administered morphine causes a significant shift of the mechanical threshold and a significant decrease in thermal latency in the rat (A. S. Advokat, Brain Res., 376: 115-118, 1986). Antibody to substance P (AntiSP) is long-lasting (i.e. > 60 min) and was antagonized by systemic naloxone (0.4 mg/kg). We have used systemic naloxone for the antagonist of this action in the present study.

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The rat oral-facial response to maxillary tooth pulp stimulation (TPS) is the jaw-opening reflex (JOR) which was utilized in the present study as a nociceptive paradigm. The digastric (a jaw opening muscle) electromyogram (dEMG) threshold (dEMGTH) and amplitude (dEMGA) were utilized as indices of nociception. Simultaneous recording of the mean arterial pressure (MAP) was also performed. Both clonidine and morphine dose-dependently and significantly elevated the TPS-dEMGTH and depressed the TPS-dEMGA. Sustained depressor conditions were induced by both agonists; however, a transient pressor response was elicited by clonidine, while a transient depressor response was elicited by morphine. Yohimbine (an alpha, adrenoceptor antagonist) but not prazosin (an alpha, adrenoceptor antagonist) or naloxone (a mu receptor antagonist) antagonized the antinociceptive effects of clonidine. Conversely, naloxone antagonized the antinociceptive effects of morphine. However, yohimbine did not antagonize the depressor effect of morphine, which was further antagonized by naloxone. The antinociceptive actions of morphine and clonidine did not include direct effects on the digastric muscle or the neuromuscular junction. It is concluded that morphine's antinociceptive effects are mediated by central mu receptors that do not appear to involve alpha, adrenoceptors. (Supported in part by grants from NIDA (DA-00418)).

440.16 LOW FREQUENCY ELECTRO-ACUPUNCTURE ANALGESIA IN RATS: EVIDENCE FOR ENDOGENOUS OPIOIDS INHIBITING EFFECTS D.E. Kellstein and D.J. Mayer, Dept. of Physiology, Medical College of Virginia, Richmond, VA 23298.

Low frequency electro-acupuncture (EA) analgesia in rats is thought to be mediated by endogenous opioids because naloxone antagonized 2 Hz EA analgesia and partially reversed 2-15 Hz EA analgesia (Watkins et al., Brain Res. 327, 1985). We conducted experiments with members of Han's laboratory in the U.S. and in their laboratory in China using the exact methods of Han et al. (measurement of tail flick latencies at the end of each 10 min period of 1, 2, or 3 volts EA stimulation of the hindlimbs, and every 3 min for 12 min thereafter). In no experiment was opiate antagonist reversal or reduction of EA observed. More specifically, we found the following: 1. Naloxone (0.02 mg/kg) and naloxone (0.02 mg/kg) potentiated 2 Hz and 2-15 Hz EA analgesia in most experiments.

2. The potentiation occurred independently of the geographic location of the experiment (China or America), the strain of the animals (Chinese or American), tail temperature, age, or weight. In some instances the females were unaffected by the opiate antagonist pretreatment while the males showed a potentiation.

3. Spinalization or DLF lesions abolished all analgesia indicating that supraspinal structures are involved in mediating the analgesic effect.

4. Hypophysectomy or adenectomy of male rats increased the level of EA-induced analgesia independently of opiate antagonist pretreatment suggesting that (a) the observed analgesia is not dependent on the pituitary-adrenal axis for expression and (b) a hormonal factor may be required. Other possible variables which have not been studied here include the effects of seasonal and circadian fluctuations. (2) EA activates an opiate system which inhibits the development of analgesia. (3) This model of analgesia is probably not an appropriate model of human EA because the high intensity of the stimulation produces perioral and facial edema that is not present in humans and because low frequency EA analgesia in humans is generally blocked by naloxone. (Supported by H.H.S. award DA 00576 to D.J.M.)

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10.17 CONTRASTING EFFECTS OF ACUTE VS. CHRONIC PROGLUMIDE TREATMENT UPON SPINAL MORPHINE ANTINOCICEPTION D.E. Kellstein and D.J. Mayer, Department of Physiology, Medical College of Virginia, Richmond, VA 23298.

Considerable evidence suggests that cholecystokinin octapeptide (CCK-8), a putative CNS neuromodulator, functions as an endogenous antagonist of opioid analgesia. Previous studies in this laboratory demonstrated that acute administration of proglumide (PGM), a CCK-8 receptor antagonist, enhances both intrathecal (IT) and periaqueductal gray (PAG) morphine analgesia (Watkins et al., Brain Res. 327, 1985), suggesting that PGM may be a useful adjunct to opioid analgesics in the management of chronic pain. The present study investigated the interaction of chronic PGM with IT morphine antinociception.

Under pentobarbital anesthesia, rats were implanted with IT catheters which terminated at the lumbar enlargement. Following a one-week recovery period, morphine (1.0 mg, IT) analgesia was evaluated weekly with a tail-flick assay during, and after, 22 daily injections of PGM (0.02 mg, IT). On day one, PGM pretreatment reliably (P<0.01, area under the curve) enhanced morphine antinociception compared to saline pretreatment, but this facilitation was absent on days eight and 15 of treatment. Following termination of daily PGM injections, morphine analgesia was unaltered by PGM pretreatment on days 29 and 36.

Weekly PGM treatment exhibited similar effects. Morphine analgesia was initially (i.e., day one) enhanced (P<0.01) by PGM pretreatment, but this facilitation was lost by day eight and remained absent for the duration of the study (i.e., up to day 36). However, inhibition of morphine antinociception was not observed with this treatment paradigm.

The present results demonstrate that whereas acute IT PGM administration enhances IT morphine, chronic treatment causes loss of facilitation and eventual attenuation of IT morphine analgesia. These findings suggest that CCK-8 blockade with PGM (or other CCK-8 antagonists) may not be an effective adjunct to opioids in the treatment of clinical chronic pain and may be deleterious. Although the mechanism(s) of this interaction have not been elucidated, tonically active spinal antinociceptive pathways are probably not affected, since nociceptive thresholds were unaltered in animals receiving chronic IT PGM alone. Studies are in progress to determine if a similar interaction occurs between chronic PGM and morphine in the PAG.

Supported by HHS awards DA 05316 to D.E.K. and DA 00576 to D.J.M.
440.19 POTENTIATION OF MORPHINE ANALGESIA BY D-AMPHETAMINE IS MEDIATED BY NOREPINEPHRINE AND NOT DOPAMINE. B. E. Teschmacher and G. I. Hatton. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Morphine and other opioid analgesics will raise the threshold for escape from an aversive state when delivered to the mesencephalic reticular formation (MRF). This effect is markedly potentiated by administration of low doses of d-amphetamine, which have no effect on the escape threshold (Sassen et al., Psychopharmacology 90:163-185, 1986). Because amphetamine does not act selectively on a single neural transmitter system, it is difficult to draw conclusions about the mechanism by which this potentiation occurs. In order to study the role of dopamine and norepinephrine in mediating the potentiation of morphine analgesia by d-amphetamine, the effects of both amphetamine, an indirect dopamine agonist, and nisoxetine (Lilly, Corp.), a selective noradrenergic reuptake blocker, were determined alone and in combination with morphine using the brain stimulation escape pain model.

Bipolar stainless steel electrodes were stereotaxically implanted into the MRF of male F344 rats which were then trained to turn a wheel manipulandum to escape from electrical stimulation to this brain site. Escape thresholds were determined using a modification of the psychophysical method of limits. Mephenesin had no effect on the escape threshold when administered by itself; it, however, potentiated the analgesic effect of all doses of the drugs when administered concomitantly. These findings suggest that the potentiation of morphine by d-amphetamine, which has been shown both in this pain model as well as in clinical studies (e.g., Forrest et al., New Engl. J. Med. 296:712-715, 1977), is likely due to increased noradrenergic activity and not to activation of dopamine systems.

Supported in part by NIDA grant DA 02326 and NIDA Research Scientist Award (CE) DA 00599.

441.2 DYE COUPLING INCIDENCE AMONG SUPRAOPTIC NUCLEUS (SON) NEURONS IS INCREASED BY LATERAL OLFACTORY TRACT (LOT) STIMULATION IN SLICES FROM LACTATING, BUT NOT VIRGIN OR MALE RATS. Q. Z. Yang and G. I. Hatton. Neuroscience Program, Michigan State University, East Lansing, MI 48824.

Transfer of Lucifer Yellow (LY) from one neuron to another in the SON has been shown to occur with increased incidence among oxytocin neurons in lactating, nursing mothers (Hatton et al., Neuroscience, in press). Since dye coupling is an indicator of electrical coupling, this supports the hypothesis that electrotonic conduction among SON neurons is part of the mechanism for synchronizing the burst of firing in oxytocin cells prior to the milk ejection reflex.

LOT stimulation has been shown to have predominantly excitatory effects on SON neurons, particularly oxytocin cells (Dougherty et al., Soc. Neurosci. Abstr. 1986, 12, 1256). This appears to be via mono- and di-synaptic connections from the main olfactory bulb to the SON dendritic zone (Hollowell et al., Soc. Neurosci. Abstr. 1986, 12, 1256). As part of our ongoing investigation of the SON, we noted that dye coupling tended to be more frequent in slices containing recorded neurons that were excited by LOT stimulation.

In the present study, LY was intracellularly injected into SON neurons of horizontally cut hypothalamic slices from 44 virgin female, 22 male and 27 lactating rats. Roughly half of the LY injections in each group were made without stimulating the LOT, while the other half were made after 10 min of stimulation at 10 Hz. A dye coupling index (DCI) was computed for each condition. DCI = no. of dye coupled neurons / total no. of dye injected neurons. When LOT stimulation was used, this value was 0.80 (stim.) compared to 0.43 (unstim.) for lactating rats and 0.58 (stim.) compared to 0.24 (unstim.) for non-stimulated SONs. The difference was highly significant (x^2 = 9.33, p<0.005). DCIs for males and virgins differed at p<0.01. LOT stimulation in the lactating rat produced more dye coupling by increasing the higher order coupling, i.e., the number of neurons coupled to an injected cell, rather than by merely increasing the incidence of coupled pairs. In non-stimulated slices from lactating rats 21 LY injections resulted in 16 singles, 3 pairs and 2 triplets; whereas 27 injections in stimulated slices yielded 13 singles, 6 pairs, 4 triplets, 1 quadruplet, 2 sextuplets and 1 septuplet. The distribution of cells coupled to the injected neurons in virgin, but not in lactating rats was described by the Poisson distribution.

These results suggest that the electrical coupling among SON neurons can be rapidly modified through sensory input in lactating rats.

Supported by NIH grants NS 16942 and NS 16942.
441.3 ANTEROGRADE AND RETROGRADE TRACING STUDIES OF THE MAIN OLFACTORY BULB INPUTS TO RAT SUPRAOPTIC NEURONS. K.G. Selimoth* M.G. Weiss and G.J. Hatton (SPON: G. Richmond). Neuroscience Program, Physiology Dept., College of Osteopathic Medicine, Michigan State University, East Lansing, MI 48824.

Earlier studies using either silver impregnation of degenerating terminals (J. Comp. Neurol. 1975, 161:31-56) or injections of free horseradish peroxidase (HRP: J. Comp. Neurol., 1978, 193:23-64) have indicated the existence of main olfactory bulb (MOB) input to the region of the supraoptic nucleus (SON). More recent evidence (Mollelow et al., Soc. Neurosci. Abstr. 1986, 12:1256), using the anterograde tracer Phascolus vulgaris leucococcaginin (PHAL), has shown that the SON receives a direct input from the MOB. However, while PHAL labeling consistently revealed fibers and terminals around and within the nucleus, the labeling was relatively sparse. Previous electrophysiological evidence (Yang & Hatton. Soc. Neurosci. Abstr. 1986, 12:1256) suggested that this connection should be robust (i.e., LGT stimulation activates many SON neurons). In an effort to address the disparity between data generated by these two approaches, we re-examined this connection.

Discrete pressure injections of 0.1% WGA-HRP (supplied by R.R. Misci) were stereotaxically placed in the MOB to study the anterograde distribution of the tracer which was visualized with either TMB or DAB chromogens. These injections revealed thick fascicles coursing within the ipsilateral lateral olfactory tract. As these fascicles passed caudal to the piriform cortex they thinned appreciably while continuing to run along the ventral surface of the brain. Continuing caudally, a plexus of fibers projected dorsomedially to densely label the lateral margins of the posterior one-half of the SON. Fluoro-gold (1-2% in water, supplied by L. Schumad) or rhodamine-labeled microspheres were injected into the SON to study the afferent projection to this area. This resulted in retrograde labeling of mitral cells throughout the main olfactory bulb without apparent preferential distribution. The dense WGA-HRP labeling appeared to contribute to the denser zone of the SON, confirming our observation from PHAL studies. Taken together with the magnitude of mitral cell labeling, the pattern of WGA-HRP labeling demonstrated a relatively sparse and indirect input into the SON. These findings are in better agreement with our earlier electrophysiological findings. Supported by NIH grants NS01410 and NS01492, BSR Postdoctoral Fellowship NS 08125 to MGM and Medical Scientists Training Program fellowship to KGS.


The SON produces the hormones oxytocin and vasopressin. Increased hormone demand, such as occurs during parturition and lactation induces ultrastructural modifications which include a) increases in soma-somatic appositions, b) dendritic bundling and c) the appearance of new dendritic synapses. Recently observed in maternally behaving virgin females has been an increase in the area of the dendritic zone (Sale et al., Soc. Neurosci. Abstr. 1985, 1, 621). The present study examined the area of the SON dendritic zone for changes in response to stimulation conditions associated with active motherhood. Exenuated dendritic bundling in the entire mediolateral extent of the zone, and investigated the possibility of differential responses within the zone. Ultrastructural analyses were made on 60 dendritic zones from virgin females, prepartum, postpartum, 10 day lactating, and 30 day postlactating rats (n = 6 per condition except in prepartum in which n = 5). Montages of the entire dendritic zones were constructed, and then area measurements of the total zone comprised of dendritic profiles, and of the total zone area (the dendritic region, plus the ventral gial lamina; VGL) were obtained. For the purposes of analysis the dendritic region was partitioned into 3 non-overlapping compartments (medial, intermediate, and lateral) of equal size. Photographs of each compartment were used for analysis of the number of dendrites and the extent of dendritic bundling. Data were analyzed with parametric procedures with significance set at p<0.05. Area measurements of the montages revealed increases in the extent of a) dendritic region, b) VGL, and c) total area of the zone in the postpartum group compared to virgins and prepartum animals. Examination of the dendritic region at higher magnification revealed increases in the proportion of bundled dendrites in the intermediate and lateral compartments in both postpartum and lactating animals as compared to virgins. Furthermore, in all treatments, the lateral compartment contained significantly more dendrites than did the most medial compartment of the zone. The increase in the size of the lateral compartment is consistent with the idea that animals is coonvent with increases in dendritic bundling; a situation in which the dendrites should actually occupy less area. These increases are most likely due to increases in the size of individual dendrites. Taken together these data confirm further demonstrate the role of increased dendrites and glia in response to gestation, parturition and lactation. Supported by NIH grants NS 10942 & NS 10910 and by a fellowship from the Medical Scientist Training Program to KGS.

441.5 CNS PLASTICITY AND BEHAVIORAL DEFICIT FOLLOWING PHOTOCHEMICAL INFARCTION OF THE WHISKER CORTICAL BARREL-FIELDS. B.E. Hurwitz, W.D. Dietrich, P.M. McCabe, B.D. Watson* and N. Schneiderman. Depts. of Psychology and Neurology, University of California, Berkeley, CA 94720.

In previous research we have used a noninvasive photochemical reaction to induce focal neuronal damage leading to infarction of the primary whisker cortical barreis in adult rats (Watson, et al., Soc. Neurosci. Abstr., 1986, 12:1256). The present study was undertaken to determine whether a behavioral task requiring detection of whisker deflection would be disrupted after sham or unilateral photochemical cortical barrel-field infarction. Food-deprived adult male Wistar rats (N=14) were trained in the alley track and further demonstrated absence of local learning throughout the testing period.

441.6 ALTERED PATTERNS OF CEREBRAL CORTEX LATERALIZATION IN MALE RATS WITH INCREASED LEVELS OF ENVIRONMENTAL COMPLEXITY. J. C. Lin*, E. R. Greer*, M. C. Diamond, Dept. of Psychology- Anatomy, University of California, Berkeley, CA 94720.

In general, the right cerebral cortex of the male, Long-Evans rat is thicker than the left in animals living in standard colony conditions (10 rats/small cage, 32x22x20 cm) (Diamond, M. C., Cerebral Domains, Eds. Geschwind, N. and Galaburda, A. M., Harvard Univ Press, 1983). More recent results (12 rats/large cage, 70x70x46 cm plus "toys", changed twice weekly) and/or a high population density (36 rats/large cage), two left cortical areas became consistently thicker than the right, thus reversing the right dominance pattern. Transverse, frozen, 40 µm sections were cut from the frontal, somatosensory and occipital cortex and stained with a modified thionine stain. From microslide projected images, cortical thickness measurements were taken on the brains from animals living in their various environments from 60-66 to 90-97 days of age. Area 18, a visual association, medio-occipital cortical region, showed significant left dominance (1-3%, p<0.05-0.01) in those animals living in enriched and enriched/high population density environments. In adjacent area 17, primary visual cortex, the usual right dominance pattern was eliminated. In area 9, a general sensory region, the left cortex was thicker than the right by 2-5% (p<0.05-0.005) in the same two living groups. In other words, the enriched and enriched/high population density experiments altered laterality patterns. The significance of these changes is yet to be demonstrated.
441.7 THE INFLUENCE OF TEMPERATURE VARIATION ON ACTIVITY-ASSOCIATED ULTRASTRUCTURAL PLASTICITY IN TETANICALLY STIMULATED NODULES OF RAVENII, C. Wurtz and R. Ellisman (UNM). Lab. for Neurocytology, Dept. Neurosciences, UCD, Los Alamos, NM 87545. We have previously reported a change in morphology of the amphibian node of Ranvier that is associated with repetitive action potential propagation (Wurtz & Ellisman, J. Neurosci., 6:1188, 1986). The present experiment was designed to test the effect of a number of extracellular vesicles within the paranodal apparatus. It is reversible and is seen regardless of the method of fixation employed. The morphological alteration is closely correlated with a reduction in the condución velocity, and a depression of the amplitude of the compound action potential (CAP). Such perturbations in CAP conduction parameters are like those observed in intermittent conduction of myelinated axons (Barron & Matthews, J. Physiol., 73:875, 1935). Utilizing a hybrid physiological-morphological experimental preparation, we have now assessed both the conduction properties of the CAP and the associated structural changes in the node over a range of temperatures. We find that CAPs recorded from intact dorsal roots exhibit normal physiology at 25°C and can be activated for extended periods with much lower vacuolization than occurs at temperatures below 20°C. Reducing the temperature to 7°C, however, causes a substantial depression in CAP conduction properties which is associated with a degree of paranodal disruption far in excess of that noted at 17°C. Post-stimulation recovery is also influenced by the temperature of the preparation. In these cases stimulation at 7°C followed by recovery at 17°C completely prevented the paranodes from regaining control morphology, if however the bath was warmed to 17°C, nodes were able to partially recover. These results are in accord with those of the early physiologists on whole-nerve condución, and suggest that impaired CAP amplitudes and velocities, and increased vacuolization which occur at low temperature may be due to the suppression of temperature sensitive metabolic activities.

Finally, data obtained from voltage clamped amphibian nodes have been held to be non-extrapolatable to mammalian processes because such nodes will not produce action potentials above 30°C. However, we note that intact fiber bundles conduct at 32°C with an enhanced effective conduction velocity. We speculate that this disparity in temperature effects between dissected and intact nerves may be due to a partial replacement of a structural complex such as the reticulum of extracellular fibrils which are found connecting structures within the nodal gap to the extracellular matrix. Damage to such delicate structures would be unavoidable given the methods for single fiber dissection.

441.9 FURTHER ANALYSIS OF THE DISRUPTIVE EFFECTS OF CHRONIC DIAZEPAM ON RECOVERY OF FUNCTION AFTER BRAIN DAMAGE. T.D. Hernandez, J. Kiefel*, M.D. Lindner, G. Knight, and T. Schallert. Department of Psychology and Institute for Neurocytology, Gallblad, CA 90520. A regimen of certain anticonvulsant drugs can severely and chronically disrupt recovery from behavioral asymmetries caused by unilateral neocortical damage (Watson, C.W. & M.A. Kennard, J. Neurophysiol., 8:321, 1945; T. Schallert et al., Brain Res., 370:373, 1986). To determine whether there might be a sensitive postoperative period during which recovery is especially vulnerable, the postoperative status of a 7-day regimen of diazepam (or vehicle control) was varied systematically in rats with unilateral lesions of the anterior medial cortex (or sham operations). Behavioral asymmetries were assessed in detail and quantified using somatosensory extinction procedures (described in Schallert et al., 1987), and forelimb placing tests. Diazepam interfered with recovery from asymmetry recorded in the somatosensory extinction tests. Under the conditions of this experiment there appeared to be a definable period of sensitivity to diazepam extending up to 120 hrs after the damage. Variability in the duration of this period may reflect individual differences in the onset of behavioral recovery. Diazepam did not affect recovery from the forelimb placing deficits. Perhaps restoration of normal placing reactions depends significantly on motor learning, and may be one of several factors that can limit susceptibility to the detrimental effects of diazepam. Suggested mechanisms and data from several ongoing experiments will be discussed, including the effects of co-administration of diazepam and benzodiazepine antagonists, partial benzodiazepine effects and prazosin (an alpha antagonist at convulant vs non-convulant doses), or the effects of the glucocorticoid dexamethasone on recovery from unilateral damage.

Supported by grant NS 33964 to T. Schallert and AA 06761 to W.W. Spindrus and T. Schallert.

441.10 REORGANIZATION OF THE SEROTONERGIC PLEXUS OF THE AREA DENTATA AFTER LESIONS OF THE MEDIAN RAPHE NUCLEUS. J.H. Haring. Department of Anatomy and Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104. The serotonergic (5HT) plexus of the area dentata (AD) arises from neurons of the median raphe nucleus (MRN) and the dorsal raphe nucleus (DRN). The projections of the MRN and DRN are topographically distributed in the AD, with the DRN primarily supplying the molecular layer and the MRN terminating in the molecular layer of the hippocampus (Azmitia, E.C. and Segal, M.J., J. Comp. Neurol. 179:961, 1978). Lesions of the cingulum bundle induce the egress of 5HT fibers in the hippocampus which result in the loss of 5HT plexus in the hippocampal formation (Zhou, F.C. and Azmitia, E.C., Brain Res. 337:331, 1986). The present study reinvestigated these neuroanatomic observations which demonstrate that indolamine-specific neurotransin lesions of the MN induce a proliferation and migration of 5HT axons remaining in a modified 5HT plexus in the area dentata. Adult, male Sprague-Dawley rats amnesthetized with sodium pentobarbital received stereotactic injections of 5,7-dihydroxytryptamine in the MN. The AD of these animals was studied using 5HT immunocytochemistry. After sectioning of the midline, the 5HT plexus of the molecular layer is normal, but the 5HT innervation of the hilar region is greatly diminished. Six weeks after lesion, the density of the hilar 5HT innervation remains less than normal, but a proliferation of 5HT fibers is seen in the molecular layer immediately adjacent to the granule cells. Not only do the 5HT fibers seem to be more numerous, but the caliber of these axons appears to be greater as well. Distinct 5HT axons arises from the inner molecular layer through the granule cell layer to the hilar region where they ramify to innervate the hilar region immediately adjacent to the granule cells. Eight weeks post-lesion, the 5HT axons have essentially normal although some enlarged varicosities are still seen. No large reactive 5HT fibers are present, and the distribution of the 5HT plexus is comparable to that of control cases. At 12 weeks, the morphology of hiliar 5HT axons is generally normal, but bulbous, intensely 5HT-immunoreactive structures similar to those seen in the hippocampus were observed in the AD. 128 degenerating axons observed 2 weeks post-lesion are seen in the molecular layer. By 20 weeks, the distribution of 5HT fibers and boutons in the hilar region is normal, but the density is still somewhat diminished. By contrast, the molecular layer is almost devoid of 5HT fibers. The present study failed to demonstrate a shift in the terminal distribution of 5HT fibers from the DRN after MN lesion. That the hilar region is preferentially affected by the expense of the molecular layer further suggests the existence of a special relationship between hilar neurons, particularly basket cells, and the 5HT input to the AD.
Detailed microelectrode maps of the SI vibrissal zone were derived in a series of normal adult rats in which the tips of subsets of vibrissae were coupled to promote their predominantly simultaneous stimulation. Studies were designed to explore the possibility that this coupling was sufficient to create a non-simultaneous stimulation of vibrissal accounts, in part, for their functional representational segregation.

Several dozen rats were subjected to microelectrode experiments in which the tips of one or more small groups of vibrissae (usually pairs) were coupled together by the application of a thin layer of methyl cyanoacrylate gel. The remaining vibrissae were clipped in some rats, in others only unclipped. In control experiments in this series, one investigator prepared the rats and clipped all vibrissae to an equal length prior to the day of the experiment, while a second investigator mapped evoked potentials in the vibrissal representation zone. As judged by the identification of the long and coupled vibrissae, blind control experiments were also conducted; in these studies, all vibrissae were left intact and undisturbed. All experiments were conducted in ketamine-anesthetized rats. Bavolek et al. (1984) used 150 micromicroelectrodes to parallel penetrations were introduced into the vibrissal representational zone in most experiments. Vibrissae effective for evoking a response of multunit clusters were defined by their predominantly simultaneous stimulation with multunit and paired receptive-field responses. As judged by projection depth, receptive fields were defined within granular and supra-granular cortical layers.

In most but not all cases, there was a substantial enlargement of the functional cortical representations of long vibrissae of rats maintained in a tactually rich environment, after all other vibrissae were trimmed for only a few days. There was also a higher probability that coupled vibrissae would be represented together at sampled cortical loci. To site a representative example, in an adult rat in which most of the vibrissal representation map was defined by their careful manual manipulation with fine glass probes. As judged by parallel penetrations were introduced into the vibrissal representational zone in most experiments. Vibrissae effective for evoking a response of multunit clusters were defined by their predominantly simultaneous stimulation with multunit and paired receptive-field responses. As judged by projection depth, receptive fields were defined within granular and supra-granular cortical layers.

The barrel fields of these rats are being histologically reconstructed so that they can be related to the functional cortical map. It is not yet clear to what extent these differences of differential behavioral stimulation of a small vibrissal subset may result in a mismatch between the anatomically constant layer 4 barrel array and the physiologically manifested territories of representation of the vibrissae in these adult rats. These results support the hypothesis derived in earlier studies in monkeys that, considered in detail, cortical representations and receptive fields are shaped by differences in the magnitudes of inputs, and by the time structure of inputs.

Supported by NIH Grants NS-10144 and NHLBI.


Previous demonstrations that experience can alter dendritic morphology in cerebellar cortex have left open the issue of whether motor learning or repetitive motor exercise is primarily responsible (Pye & Welsh, 1979, Science, 206, 230; Floeter & Greenough, 1979, Science, 206, 43). On the 11-month-old Long Island hooded female rats were randomly assigned to one of four experimental conditions balanced for litter: Individual Conditioning (IC), in cages, Acrobatic Condition (AC), with access to a running wheel. Body weight, food consumption, and spatial maze, and required the rats to traverse obstacles such as narrowed balance beams, swinging bridges, ropes and chains, and teeter-totters. The FX rats were given progressively longer trials per day that became progressively more difficult over the 30 days. The pyramidal cells were divided into two subtypes based upon their preferred or nonpreferred paw (33 reach sessions over 16 hemispheres with all of the "nontrained" hemispheres. Significant differences were found in the total dendritic length and number of branches were greater in the MFS condition to the trained paw then in the hemisphere opposite the nontrained paw. These within-animal differences were greatest in rats trained to reach with their nonpreferred paw. This study investigates subtypes of another cell population within this region. The MFS sample region was the area of highest uptake of 2-DG in animals which were injected prior to performance of the reaching task (Fuchs, et al., Soc. Neurosci. Abstr., 5, 294, 1983). Basilar branches of pyramidal cells in layers IV/III were traced using a computerized tracking system. The pyramidal cells were divided into two subtypes based upon morphological differences; the first type having a forked apical dendritic shaft, the second type having a simple primary apical shaft. We observed that the "forked" subtype of cells are typically located higher in the cortex than are the single shaft cells, suggesting that these cells may be primarily layer II cells. The data presented below compare all of the "trained" hemispheres with all of the "nontrained" hemispheres.

The pyramidal cells in AC rats were approximately 6% thinner than in the other three groups, P<.01. Between about 50% of wheel running and cerebellar cortical thickness were not correlated in the VX rats (Spitzer et al., n.s.), also indicating that motor exercise alone does not affect cell size.

MEAN THICKNESS OF MOLeCULAR LAYER (microns), VERMIS + LATERAL

<table>
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<tr>
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<th>IC</th>
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<td>140</td>
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Supported by NIMH 35321 and the Retirement Research Foundation.
PLASTICITY OF INNERVATION OF SUPERIOR COLLICULUS BY RETINAL TRANSPLANTS. J. Radek1, L. Qavi2 and R.D. Land1.


Retinal ganglion cells innervate the most superficial 400-500 μm of the contralateral superior colliculus (the superficial gray and the optic laminae) in normal rats. In the presence of this normal retinotopic input, embryonic retinal tissue transplanted to the dorsal surface of the midbrain in neonatal rats survives, differentiate and innervate the superior colliculus with a projection restricted to its dorsal surface. We have examined how this pattern of innervation, which is established within a month of age, can be modified by depriving the superior colliculus of its normal optic input.

Embryonic (E14) rat retinae were transplanted above one superior colliculus of neonatal (P1) rats. Experimental animals received a unilateral enucleation at its dorsal surface. We have examined how this pattern of innervation, which is different from that of the normal contralateral superior colliculus, which is restricted to the superficial gray and the optic laminae, can be modified by depriving the superior colliculus of its normal optic input.

The enucleation resulted in the substantial innervation of the superficial gray lamina by transplant axons. These axons possessed elaborate arborizations, resulting in a fine, granular projection throughout this lamina. Electron microscopic analysis revealed that the superficial gray lamina, the normal termination zone for retinal ganglion cells, contained many transplant-derived pre-synaptic profiles that were seen projecting to the contralateral superior colliculus, in which the normal visual projection remained intact. These fibers, as in controls, were confined to the surface of the colliculus, and transplant-derived pre-synaptic profiles were similarly restricted. These results indicate that depriving the superior colliculus of its normal optic input prevents fibers from those transplanted retinae from entering the contralateral superior colliculus, resulting in a fine, granular projection throughout this lamina.

The presence of c-fos protein-like immunoreactivity in the mature mammalian brain, together with its rapid induction during synaptogenesis, suggests that the c-fos proto-oncogene is involved in plastic changes in the mature nervous system.

We thank the Medical Research Council of Canada for support and Dr. Tom. Curran (Roche) for providing anti-fos serum.

LOCALIZATION OF GAP-43 (B-50) IN THE RAT BRAIN. L.I. Benowitz, L.P. Finkelnburg, M. Dragunow, and Dr. Tom Curran. Department of Pharmacology, Faculty of Medicine, Dalhousie University, Halifax, N.S., Canada B3H 1Y4.

Using immunohistochemical techniques we have recently shown that the product of the c-fos proto-oncogene, the c-fos phosphoprotein, is confined to the adult mammalian brain (Dragunow et al., J. Neurosci., 15:113, 1987). Furthermore, the immunostaining is found predominantly within the nuclei of nerve cells in distinct layers of the spinal cord, lateral geniculate and visual areas, the hippocampus and entorhinal and piriform cortices and granule and Purkinje cells in the cerebellum. Axons and dendrites of nerve cells are not stained. Pre-absorbing the c-fos anti-serum with the peptide that is recognized by the polyclonal antibody results in almost complete abolition of the immunostaining in the dentate gyrus. This work was supported by NIH grants EY05962 (JDK) and EY05283 (RLD).

441.15 DISTRIBUTION AND INDUCTION OF c-fos PHOSPHOPROTEIN IN ADULT MAMMALIAN BRAIN. M. Dragunow and H. A. Robertson. Department of Pharmacology and Therapeutics, Univ. of Calgary Med. Sch., 3330 Hospital Dr., NW7, Calgary, Alberta T2N 1N4.

The product of the c-fos proto-oncogene, the c-fos phosphoprotein, is present in the adult mammalian brain (Dragunow et al., J. Neurosci., 15:113, 1987). The induction of c-fos protein in neurons of the central nervous system results in the appearance of the c-fos transcript and an increase in immunoreactivity with the antibodies raised against electrophoretically pure GAP/B-50, the other induction of the c-fos protein-like immunoreactivity in brain tissue. GAP/B-50 immunostaining was restricted almost exclusively to the neocortex, but was also noted in the staining of various nuclei of the basal ganglia, thalamus and of different cytoarchitectonic areas of cortex.

The c-fos protein-like immunoreactivity in the mature mammalian brain, together with its rapid induction during synaptogenesis and high frequency electrical stimulation suggest that the c-fos proto-oncogene is involved in plastic changes in the mature nervous system.

We thank the Medical Research Council of Canada for support and Dr. Tom. Curran (Roche) for providing anti-fos serum.


We have investigated the conditions under which the proto-oncogene c-fos is expressed in neurons of the rat spinal cord and hippocampus. The expression of the c-fos proto-oncogene and prolonged changes in excitable cells (Glowatz, P. et al., Nature 322: 619, 1986), we looked for evidence that the c-fos protein produced in spinal cord neurons following sensory stimulation, as sensory stimuli which activate small diameter afferents produce long term changes in dorsal horn neurons (Wall, P.D., Phil. Trans. Roy. Soc. B (Lond) 308: 395, 1985). All experimental procedures were performed in anesthetised rats (5% halothane, 70 μm, with TMB method or at 70 μm, with nickel-intensified DAB reaction for electron microscopy. For lesioned brain were cut at 40 μm and reacted by the TMB method or at 70 μm, withalternates sections reacted by the TMB method for light microscopy and the nickel-intensified DAB reaction for electron microscopy.

For HRP-injected transplants, coronal sections of the transplantation. The host was perfused 24 hours after HRP application or 72 hours post-seizure the number of granule cells showing positive immunostaining is found predominantly within the nuclei of nerve cells in distinct cortical layers, hippocampal pyramidal cells, dentate granule cells and cells in the striatum, amygdala, entorhinal and piriform cortices and granule and Purkinje cells. In the cerebellum, axons and dendrites of nerve cells are not stained. Pre-absorbing the c-fos anti-serum with the peptide that is recognized by the polyclonal antibody results in almost complete abolition of the immunostaining in the dentate gyrus. This work was supported by NIH grants EY05962 (JDK) and EY05283 (RLD).

Injection of mustard oil into the medial gastrocnemius muscle resulted in the appearance of labelled nuclei within lamina I, II and IV. Expression of the proto-oncogene was not noted in the regions of the spinal cord such as the gracilis or ventral horn which receive direct inputs from low threshold large diameter afferents.

In contrast, -12 h following stimulation of the perforant pathway (250 Hz, 200 ms) to generate long term potentiation in dentate gyrus, no induction of p55 c-fos was observed. However direct perfusion of the dentate gyrus with 10 μm calcium, 10 μm carbamol or electrical stimulation of CA3 did result in a massive expression of the proto-oncogene within bilateral dentate gyrus. Thus p55 c-fos can be induced in neurons of the CNS by a variety of stimuli, but synaptic induction of the gene has only been observed in the spinal cord where it may indeed be related to synaptic reorganisation.

The induction of c-fos protein in neurons of the central nervous system is widespread and appears to be a general mechanism for induction of new synaptic contacts following stimulation of the nervous system. In contrast, no c-fos gene expression has been observed in the spinal cord of rats which were stimulated with a low threshold volley of nerve fibers which are associated with increased excitability, but are not associated with induction of new synaptic contacts. The induction of the c-fos gene occurs in numerous areas of the brain, including the neocortex, the hippocampus, the caudate-putamen, the amygdala, the thalamus and of different cytoarchitectonic areas of cortex.

This distributional pattern raises the question of whether the c-fos gene expression in all areas containing high levels of GAP-43/B-50 are caused by synaptic changes, or whether the protein may function in at least some of these areas in some other capacity (e.g., general signal transduction).

Supported by the Canadian Institutes of Health Research and the American Heart Association.

The growth-associated protein GAP-43, a protein that has been associated with the development of synaptic relationships, has recently been shown to be equivalent to phosphoprotein B-50 (Fl), a C-kinase substrate which has been implicated in phosphoinositide metabolism and in the events underlying long-term synaptic potentiation. Thus, although most neurons cease expressing GAP-43 at high levels after the establishment of stable synaptic relationships, its continued expression in a select subset of neurons may confer specialized properties upon these cells' synaptic endings, perhaps related to ongoing functional plasticity. To identify such areas in the human brain, we used a full-length cDNA probe to the human gene (Nove et al., Mol. Brain Res., in press) in Northern blots to examine levels of GAP mRNA in different areas of embryonic and adult human brain. In 32- week fetuses, levels of the 1.6 kb GAP mRNA were extremely high in all brain regions examined, consistent with the idea that all neurons express high levels of the protein when synaptic relationships are being established. In four adult brains, however, levels of GAP mRNA showed a highly reproducible, striking regional heterogeneity. Levels of GAP mRNA were extremely low in thalamus, caudate-putamen, and in sensory and motor areas of the neocortex (e.g., Brodmann Areas 17, 18, and 41). However, higher intensity areas of the cortex showed very high levels of GAP mRNA, including the inferior temporal region (Areas 20, 21), frontal cortex (Areas 10 and 11) and periventricular structures (Areas 40 and 44). Levels of GAP mRNA were modest in the cerebellum and low in the hippocampus as a whole, although preliminary in situ hybridization studies suggest that specific neural populations in the hippocampus do express the gene. Controls included use of other genetic probes (e.g., cDNAs for microtubule associated proteins and glyceraldehyde 3-phosphate dehydrogenase) that showed equal distribution in all areas. These results suggest that, by virtue of their expression and their mature expression in the GAP gene, a subset of neurons in associative areas of the brain may be specialized for ongoing synaptic plasticity.

Supported by the National Down Syndrome Society (to RD), the NIH (HD35690 to LS; NS/NS 31562 to EBD), and the American Heart Association (to SFP).

DEVELOPMENTAL DISORDERS

441.1 AMPHETAMINE-INDUCED BEHAVIOR IN RATS TREATED WITH PHENYLACETYLATE (PXA). D. H. Heilig (Neurology Lab, University of Rochester, Rochester, NY State). Supported by the National Institute on Drug Abuse, Baltimore, MD 21224.

Phenylacetate (PA), a major metabolite of phenylalanine, may be the primary cause of brain dysfunctions associated with the inherited disorder phenylketonuria. PA is metabolized by phenylalanine hydroxylase, the enzyme responsible for conversion of phenylalanine (PHE) to tyrosine, an essential amino acid. Left untreated PKU results in serious mental retardation and a high incidence of epilepsy. The treatment of PKU patients includes administration of low PHE for receptors on a common transport system and thus reduce entry of PHE into brain cells, and additional dietary restrictions. The only treatment is severe dietary restriction of PHE beginning early in life.

Recent reports suggest that adverse behavioral changes and impaired performance are consequences of elevated serum PHE even in early treated PKU subjects, whether from deliberate termination of diet or from chronic failure to adhere to the strict regimen. We reported earlier that administration of amphetamine, isoxazoline, and valine (VIL) to PKU patients improved neuropsychological performance, particularly in tests of abstract reasoning and cognitive functioning. VIL concentrations in CSF were reduced after VIL treatment that VIL was not affected, suggesting that these amino acids may successfully compete with PHE for receptors on a common transport system and thus reduce entry of PHE into brain cells.

A multicenter, double-blind trial of effects of VIL counter-treament in adolescents and young adults has been completed. Fifteen subjects, both on and off diet, received either VIL or a control mixture of amino acids (CS) in a random sequence for four periods of 3 months each. Tests were administered before and at the end of each period.

During VIL periods, time to completion of the Attention Diagnostic Method was faster, showing shorter latency; reaction time for the Cognitive Performance Test approached significance, and significantly fewer errors occurred on a sensory-perceptual task (finger tip writing).

Serum PHE concentrations were similar throughout the study. No adverse effects were noted. These data support our hypothesis that attention based performance during VIL periods was superior to either baseline or CS periods, and that VIL promotes improved central nervous system functioning even in the presence of high concentrations of PHE.

Restricted subdivisions of the mesencephalic projection contain severely reduced dopamine (DA) levels in striatum of the mutant mouse weaver. The anatomical pattern of loss is along functional lines so that the ventral striatum is much more affected than the dorsal striatum. The DA deficiency occurs postnatally and is not fully expressed until 1 month. DA content and the typical islandic distribution of Fos-like immunoreactivity (F-LI) in the striatum appear normal at postnatal (P) day 7. We and others have observed partial depopulation of the weaver SNpc (cell group A9) and whether the magnitude of the deficit for the time of appearance of this deficit has been determined. We report here the first part of a quantitative study aimed at identifying which subpopulations of dopamine neurons in the midbrain are vulnerable and when during development the cellular defect appears.

Transverse 20 or 30 μm sections through the midbrain of a weaver homozygote and littermate control at each of 3 ages (P7, P21, adult) were stained for TH-IR immunoreactivity. The numbers of TH-IR neurons in the midbrain are decreased. The quantitative measurements suggest the following conclusions: (1) At adulthood there is a deficit in absolute numbers of TH-IRs in the cell groups A9 and A10 of weavers (45% control). (2) The loss is strongly expressed at P21 (weaver 80% control). (3) The loss appears spatially restricted (distal distribution curves for weaver and controls at P7, P21 and maturity differ most in the middle part of the transverse dimension). (4) Since the islaemic system, after P10, disappears in the weaver after P21, the loss of cells occurring between P21 and maturity must include the cells giving rise to the dopaminergic system. The finding that most mesencephalic DA neurons are in place in the newborn weaver suggests that the deficit is not primarily one of initial migration and initial presentation of the cerebellar defect in weaver. However, we have also found that at P7, the capacity of striatal synaptoneurosis to accumulate [113]HDA is already reduced by 50% in weaver. Thus, since significant cell loss is detectable in total midbrain TH-IRs, the weaver disease is detectable biochemically. Supported by NS20181 and MB00655.


Border disease (BD) of sheep is a teratogenic disorder caused by a virus in the genus Pestivirus (Togaviridae). When acquired congenitally, the virus causes marked demyelination throughout the CNS with an accumulation of abnormal glial cells in white matter regions. Infection is spread by the ingestion of infected lamb's milk.

Pregnant ewes were inoculated with BD virus at 30 days gestation and myelin proteins were quantitated in several regions of the CNS during prenatal and postnatal development for comparison to age matched controls. Myelin basic protein (MBP), proteolipid protein (PLP), and myeloblast-associated glycoprotein (MAG) were measured by densitometric scanning of Western blots and/or radioimmunoassay. The specific activity of [3H]-cyclic nucleotide-3'-phosphodiesterase (CNPase) was measured enzymatically.

Deficiencies in the myelins proteins were detected as early as 16 days of gestation. At all ages examined, the deficiencies of myelin proteins were most pronounced in the cerebellum. In six month old infected lambs, MBP and PLP of the cerebellum were between 40 and 50% of controls. Deficiencies in MAG and CNPase were between 70 and 80% of control. Similar results were obtained for the corpus callosum and spinal cord of infected lambs, but the deficiencies of myelin proteins were not as great. A common finding in all regions examined was that MBP and PLP were reduced more than NCG or CNPase.

These results are probably explained by a greater deficit of compact myelin in which MBP and PLP are localized, than of associated oil droplets or membranes in which NCG and CNPase are concentrated. Similar results have been obtained in several demyelinating mutants pointing to common factors in viral and genetically caused hypomyelination.

442.5 DORSAL ROOT GANGLION NEURONS FROM NORMAL AND TRISOMIC (21) HUMAN FETAL TISSUE SHOW TETRODOTOXIN-SENSITIVE AND -INSSENSITIVE TETRODOXOTIN-SENSITIVE INHIBITORY CURRENTS AND A SLOW SODIUM CURRENT. J.T. Concannon and P. Damski*, Dept. of Pharmacology, Southern College of Osteopathic Medicine, North Miami, FL 33160.

Recently, it has been shown that d-amphetamine is able to reverse 6-hydroxydopamine-(6-OHDA)-induced T-maze deficits in developing rats, and that this effect was in turn reversed by pre-treatment with the dopamine (DA) receptor blocker haloperidol (Concannon and Damski, 1987), suggesting that amphetamine works by a DA mechanism. Others (Helffer and Seiden, Brain Res., 1982 244:81) have suggested that the effects of d-amphetamine in 6-OHDA-Treated rats are due to serotonin (5-HT) mediation, since they were blocked by pretreatment with methysergide, a purported 5-HT antagonist, although learning was not measured in this study. Hence, methysergide may also modulate amphetamine's effects on T-maze learning.

In the attempt to test this notion, we utilized intracranial injections of 6-OHDA (150 μg) or its vehicle in 3-day old rat pups after desmethyldaprenaline (DHE) pre-treatment and examined T-maze learning at 20-23 days of age. At 60 min prior to testing, animals received either no injection or methysergide at one of 3 doses (1.0, 4.0, or 8.0 mg/kg). At 30 min prior to testing, animals received either saline, d-amphetamine (0.5 or 1.0 mg/kg), or fenfluramine (9.0 mg/kg). Hence, the experiment was a 4 x 2 factorial combination of methysergide dose (none, low, intermediate, high), drug treatment (saline, low amphetamine, high amphetamine and fenfluramine), and brain status (6-OHDA vs. vehicle). Animals were then trained for 20 trials in a standard T-maze position discrimination task.

The 6-OHDA treatment produced a T-maze learning deficit that was reversed by d-amphetamine, although methysergide was ineffective in normal rats. Fenfluramine, on the other hand, was ineffective in 6-OHDA-treated rats, but produced a methysergide-reversible learning deficit. The low dose of methysergide blocked the effects of d-amphetamine in 6-OHDA rats, even though it reversed 6-OHDA-induced learning deficits when administered alone prior to saline. These results indicate that methysergide together with either d-amphetamine or fenfluramine improved learning in 6-OHDA rats, even though they were not beneficial when administered in combination as an "agonist" at higher doses. However, when effective, it only antagonized the effects of fenfluramine. Most importantly, methysergide in low doses seems effective by itself in reversing T-maze learning deficits and perhaps should be screened in human ADS.

(Supported by a Southeastern College of Osteopathic Medicine Research Grant.)

Mature trisomy 16 is a model of trisomy 21 in humans (Down syndrome). DRG neurons in culture contain a fast, TTX-sensitive and a slow, TTX-insensitive Na conductance. DRG neurons from T16 mouse embryos have a faster action potential, with higher rates of depolarization and repolarization and also higher fast and slow Na conductances than do normal neurons. In fetal DRG neurons the slow Na conductance is 3-4 times the fast, TTX-sensitive Na conductance. There is no available information on the properties of the slow Na conductance at the single channel level however. The purpose of this study was to characterize the single channel activity of the TTX-resistant component in trisomic and control neurons.

Single channel currents were recorded in cell-attached and in cell free (inside-out) modes, with a patch amplifier (EPC-7, List Electronic, FRG). DRG neurons were obtained and maintained in tissue culture as previously described (Orozco, et al. Dev. Brain Res. 22:111-122). Microelectrodes with 10 MΩ resistance were used. Gigaseals of 10 to 15 gΩ were obtained. Voltage membrane was varied between +40 mV to -40 mV in the cell-attached experiments, and from +120 mV to +20 mV in the inside-out experiments. The pipette solution contained (in mM) NaCl 130, MgCl2 10, dextrose 25, sucrose 10 and Hepes-HCl 10, at pH 7.4 with 10 mM TTX. The internal solution contained: NaF 28, Tris-HCl 90, MgCl2 2, TEA-Cl 5, CaCl2 1, EGTA-Tris 11 and Hepes-Tris 10, at pH 7.4. In some experiments 4-aminopyridine 7.5 nM was included.

In the cell-attached configuration, three different currents were recorded. Two spontaneous fluctuations carried inward currents, about 0.5 pA and 1.0 pA at -100 mV. An outward current also was observed when pulses were applied to the membrane, probably due to Cl-. Both inward currents decreased as the membrane potential became less negative. The frequency of the larger component (B p50) decreased in presence of 4-AP, suggesting movement of Na through a transient K conductance. The slope conductance of the smaller inward component was 2.7 pS in cell-attached configuration and 2.2 pS in the cell-free mode. The current inverted at +30 mV, close to Na equilibrium potential (Vem = +39 mV), indicating high selectivity for Na.

These results suggest the presence of a small ionic channel associated with the slow Na conductance in DRG neurons. Additional experiments are required to see if Na also can move through an additional channel.

442.8 RETAINED FETAL GROWTH OF FOREBRAIN COMMISSURES IN BALB/c MICE: MODE OF INHERITANCE. D. Wahlsten and G. Smith*. Dept. of Psychology, Univ. of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

Although only a minority of adult BALB/c mice show a marked deficit of or absence of the corpus callosum (CC), in 17 to 18 day BALB fetuses almost all show retarded growth of both CC and the hippocampal commissure (HC). An index of retardation was derived from previous data on genetically normal mice (Wahlsten, J. Comp. Neurol. 1987) in that the mean of the ratio of the total area of CC and HC to cross-sectional areas on fetal body weight. Because deviations from the control mean, or retardation, of the expected squared residual was also determined with quadratic regression. The index was then a standard score (z), the difference between actual and calculated body weight, divided by the expected residual. A z value less than -2.0 was indicative of significantly retarded commissure development. The first study compared BALB/CaH, C57BL/6J, their F1 and F2 hybrids, and backcrosses of F1 to BALB. Percentages of n fetuses in the body weight range of 0.5 to 0.8g with z scores below -2.0 were as follows: BALB/CaH (n=14), C57 (8 of 13), F1 (17 of 26), F2 (1 of 26). In F1 F2 and F2 backcrosses of retarded COCH in F1 and F2 generations did not deviate significantly from Mendelian ratios for a single gene, but there was no evidence of homogeneity in the frequency distributions. Furthermore, retardation in abnormal F2 hybrids was less severe than in BALB.

The second study examined fetuses of BALB/cByJ, C57BL/6ByJ, their F1 hybrid and seven recombinant inbred strains (C, E, G, H, I, J, K) all BALB. All had z scores below -2.0, whereas all C57 and F1 hybrid fetuses were above -2.0. Among the recombinant inbred strains, strain I showed greatly retarded to the same degree as BALB, and strain I closely resembled C57. Almost all fetuses of strains H and J were below z = -2.0, but they were not as retarded as BALB. Strains D and K showed mild retardation. On average their z score was less than 0, but only about 50% of them were actually below 0. Z values were lower in strains that have a high incidence of retared COCH in F1 and F2 generations. From these data, polygenic inheritance of the retardation in BALB is likely.

Gross anatomical differences are apparent between brains of normal adult rats and those whose mothers received an injection of methylazoxymethanol acetate (MAM, 30mg/kg on gestation day 15). MAM rats are deficient primarily with the posterior cortical area affected most. To discern if a functional difference could be mirrored in this structural deficit, the visual evoked potential (VEP) to a flash was compared for normal versus MAM rats.

Four normal and 4 MAM rats were chronically implanted with 10-12 fine stainless steel wires cortical electrodes and 2 screw electrodes as a reference and ground (sinus and cerebellum respectively). Each VEP was the average of 100 flashes (9.27 flashes/sec) given to a restricted rat; an epoch was either 280 or 440 msec. At least two sets of VEPs at 6 monopolar derivations were thus obtained at each session. The data discussed represent at least 3 such sessions per animal over a minimum period of 2 weeks. The two sites chosen for analysis generated the highest amplitude VEPs and were located at comparable points over the posterior cortex of normal and MAM rat.

Visual inspection of the VEPs showed good within-subject and within-group consistency, revealing a VEP pattern differentiating normal and MAM rats. Both normal and MAM rats contained a prominent peak around 40msec (34.3 vs 43.0), but a larger peak around 75msec (37.3 vs 79.0), and then a rising positivity which usually reached maximum around 170msec (162.4 vs 180.2); this positivity had variable multiphasic. The most salient difference was the presence of a high amplitude peak around 210msec in the normal rats which rode upon, or preceded, the preceding period of positivity; it was often enhanced at the 2.7 flash rate. In no instance was an MAM VEP seen to have this peak, although P170 was sometimes enhanced by the higher flash rate. Furthermore, overall amplitude of the VEPs in normals was always higher than in the MAM group with the latter 60 msec peak always occurring later than 100msec. In MAM rats the early component (P40) was often the largest. Between 300 and 400msec, at a flash rate of 9, prominent components mimicking the potentials seen between 100 and 200msec, but at a lower amplitude, occurred in the normals. These components were not apparent in MAM VEPs.

In conclusion, the structural abnormalities of the MAM cortex mirror themselves in functional abnormalities seen through their VEPs. Visual discrimination deficits of MAM rats may be related to the severity of loss of some of these components.

422.12 CEREBROCORICAL MICRODYSGENESIS IN NORMAL HUMAN BRAINS. W.E. Kaufman* and A.M. Galaburda, (SPONS: S. Weinerbarg). Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

We have described in four consecutively studied brains of male individuals with developmental dyslexia the presence of cerebrocortical microdysgenesis (layer I ectopias and associated dysplasias) involving perisylvian cortices predominantly in the left hemisphere (Galaburda et al., Ann. Neurol. 18:222, 1985). In this form of microdysgenesis the majority of neurons are appropriately placed in the superficial layers, however, is anomalous; neurons may be found in the molecular layer and, at times, even in the subarachnoid space; there is often distortion of the cortical curvature to form microgyria or brain warts. The anomalies in the dyslexic brains number between twenty and forty, detected after each brain has been embedded whole in celloidin, sectioned at 35μm, stained with cresyl violet, and analyzed every forty sections. The literature on these types of developmental anomalies shows that they can be found in normal brains studied at autopsy; the figure documentation is five to thirty percent of cases, with the larger figure referring to individuals of dubious normality (Veith and Schwid, Forcier. Neurol. Psychiatry. 44:1, 1976).

Most of these reports state that the anomalies are few in numbers and predominant in the right inferior frontal region. However, none of these studies examined brains at as great a level of detail as we do our dyslexic brains. Therefore, we carried out a study of ten normal brains from the Talavon Collection at the Armed Forces Institute of Pathology that have been prepared and examined in a manner identical to that of our dyslexic sample. The normality of the subjects was ascertained from available clinical records accompanying the specimens. The ages ranged from 3.5 to 87 years; there were nine males and one female. Four of these brains showed abnormalities similar in type to those of the dyslexic brains, but in far smaller numbers and in different locations; the brains showed one left frontal ectopia; a third brain showed one left temporal ectopia; the fourth brain showed one leftfrontal ectopia. Thus, our findings do not differ substantially from those previously reported. A finding of 4 out of 10 normal brains of the present form of microdysgenesis. However, unlike the finding in the dyslexic brains, the anomalies were seen in small numbers (basically isolated ectopias), and there is no particular side predilection. (Supported by NIH grant HD 19819.)

422.13 CONNECTIONAL ANOMALY IN ASSOCIATION WITH CEREBRAL MICRODYSGENESIS IN THE RAT. A.M. Galaburda, G.D. Rosen, and G.E. Sherman, Neuroanatomical Dyslexia Lab, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

We have reported cerebrocortical microdysgenesis (layer I ectopias and associated dysplasias), including at times microgyria, to be a consistent finding in the brains of male individuals with developmental dyslexia, and have suggested that these anomalies are accompanied by abnormal neuronal connectivity (Galaburda et al., Ann. Neurol. 18:222, 1985). In this type of microdysgenesis, the majority of neurons are appropriately placed in the cortical plate. The arrangement of neurons in the superficial layers, however, is anomalous; neurons may be found in the molecular layer and, at times, even in the subarachnoid space; there is often distortion of the cortical curvature to form microgyria or brain warts. The disorder, in the human, is thought to reflect a neuronal event taking place at five to six months of gestation. Similar anomalies can be induced in rodents with damage occurring near the end of the period of neuronal migration to the cortical plate.

During the course of a cerebral section study in adult Wistar rats (see Rosen et al., Soc. Neurosci. Abstr., this issue), we found that one of the animals that had had a callosotomy also showed the unusual finding of spontaneous microgyria involving the posterior-lateral neocortex of the left hemisphere. The brain had been cut in coronal sections and processed according to the Fink-Heimer method, and adjacent sections stained with cresyl violet for Nissl substance. The cytoarchitectonic anomaly consisted of a fused molecular layer containing nests of ectopic neurons, a poorly differentiated second layer continuous with layers II, III, and IV of surrounding cortex, and compressed layers V and VI consistent with curvature artifact. The antero-posterior extent of the anomaly was 900 μm. Examination of adjacent sections for terminal degeneration revealed abnormal connectivity: there were unlaminate, dense projections from the pial surface to the depth of layer II, IV, a projection-free zone corresponding to layer V surrounding this zone of projection, with occasional projection-rich bridges to a layer VI that also contained dense projections. This is consistent with the findings of caudal terminations in this region, which consists of highly laminated projections to layer I, II, and IV (skipping layer II and the subpial portion of layer I), a layer V devoid of projections, and a projection-rich layer VI. This is also in contrast to the findings in other species, which demonstrate no alterations in connectivity to the inside-out order, and are thought to reflect an earlier dysgenetic event. Anomalous projections in the present case may reflect preservation of the primitive, undifferentiated pattern of projection, with as yet unknown errors, as well as abnormal sprouting during a period of great plasticity. (Supported by NIH grant HD 20806.)

422.14 NEUROPEPTIDE ARCHITECTONICS IN THE BRAIN OF THE NEW ZEALAND BLACK MOUSE. G.L. Gudelman, G.E. Sherman, and A.M. Galaburda, Neuroanatomical Dyslexia Lab, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

New Zealand Black (NZB) mice spontaneously express autoimmune disorders (Howie and Helyer, Adv. Immunol. 9:215, 1968), developmental cerebrocortical anomalies similar to those of the dyslexic brains, and problems with learning active avoidance tasks (Nandy et al., Life Sci., 33:1499, 1985). The emerging picture is comparable to that of male dyslexics, who show similar developmental brain anomalies (Galaburda et al., Ann. Neurol., 18:222, 1985), and have learning problems particularly involving language, and, together with their families, may show increased rates of certain autoimmune and allergic disorders (Geschwind and Behan, PNAS, 79:5091, 1982). The brain anomalies in both groups consist of nests of neurons in layer I of the neocortex, predominantly on one side of the brain, with distortion of adjacent cortical layers. It is believed that these types of anomalies have their origin during the last stages of neuronal migration or shortly thereafter. Our recent work has been aimed at establishing the NZB mouse as an experimental model for the study of the developmental pathology seen in dyslexia.

Reduction in the volume of the neocortex and subcortical-parasagittal complex, and in the number of cholinergic neurons in the basal forebrain region also has been reported in the NZB brain (Zilles, Senile Dementia of the Alzheimer Type, 1985). In order to assay whether cytoarchitectonic anomalies are expressed at the level of chemical markers of fundamental specialization, we employed immunohistochemical techniques to identify vasoactive intestinal peptide (VIP), cholecystokinin octapeptide (CCK-8), and somatostatin in the cerebellar cortex of NZB and control mice. Five adult NZB mice and five DBA control mice were perfused with 0.9% saline followed by 2% paraformaldehyde/0.1% glutaraldehyde, and frozen sections were cut at 30 μm. For each neuroanatomical region, the entire cross-section of the brain was stained using polyclonal antibodies (Immuno Nuclear Corporation) and counterstained with methyl green. An additional series from each brain was stained with cresyl violet to allow for the determination of cytoarchitectonic boundaries. A Gould FD-5000 image analyzer interfaced with a VAX 11/750 minicomputer is used for measurements of cell densities. The atlas areas are parceled on the Nissl series, overlayed onto digitized immunohistochemical images, and the distance between the architeconic areal and laminar topography of each peptide and the number of positively stained cell bodies are determined. Comparisons will be presented for the experimental and control strains for VIP with no cytoarchitectonic anomalies. (Supported by NIH grants HD 19819 and 20806.)
INCREASED DOPAMINERGIC SENSITIVITY FOLLOWING PRENATAL MPTP AND MPP+ TREATMENTS IN RATS.
S.F. Caldecott*, S.<long space>15

X-irradiation of the hippocampal region of newborn rats selec­tively reduces the number of granule cells, which are born after birth in contrast to other neurons in the region. In this study we have examined the effects of such X-irradiation on the den­dritic differentiation of the residual granule cells. Further studies need to elucidate the mechanism(s) underlying the long-term receptor sensitivity changes rats pre­viously exposed to MPTP. Supported by University Research Council, Psychiatry Dept. Intramural Grant and Ommich Electric, Inc.


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We are examining infant stump-tailed macaques (M. Arctoides) reared from birth to three months of age with electronic sensory substitution devices to assess correlations between development of spatial-motor behavior and development changes in the neocortex. Groups of animals are used as follows: 1) control (no treatment with the Trisensor Aid T5A in continuous use, blind control group with 2) silent, dummy versions of the T5A or 3) sound-making but non­functional versions of the T5A were 4) sighted, colony-reared con­trols. Cage observations and tests of spatial-motor behavior are made throughout the three month post-natal period and the brains and the neocortex are examined, using Golgi-Cox, microscope, and computer (Blookquint II) techniques. We have now sampled several neocortical areas comparing patterns of development and the states that were reported for groups 1 and 2 at Neurosciences in 1985 and 1986.

Preliminary surveys have been completed in the following areas: 1) precentral motor area, 2) primary somatosensory cortex, 3) secondary somatosensory cortex, 4) inferior parietal lobule and auditory areas.

It appears that continuous input of nonrelevant device signals on noise that fails to provide useful information about the spatial environment is disruptive to both neo­cortical and spatial-motor behavioral development. These animals do not appear to compensate for the absence of meaningful input with either continuous or intermittent noise. We are examining infant stump-tailed macaques reared in a sound-making but non­functional version of the T5A.

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Among the calsequestrin induced calcium binding proteins (CaBPs), the 2SK Calbindin is found mainly in the stellate and central nervous system, where the largest concentration is by far observed in the cerebellum (Mariani et al, 1989). Using anti-CaBP antibodies have clearly showed that cerebellar distribution is restricted to the Purkinje cells (PCs), this being confirmed quantitatively by radiolabeled probe of CaBP in the cerebellum of mutant mice lacking PCs (Farber et al, Brain Res. 1989). PCs were counted in every 15th section of the sagittal plane; PCs were counted in every 15th section of the sagittal plane; PCs were counted in every 15th section of the sagittal plane; PCs were counted in every 15th section of the sagittal plane. The aim of the present study was to count the number of PCs in the cerebellum of this mutant to confirm that the raw depletion in CaBP content was not due to a decrease in the number of PCs in the cerebellum of this mutant to verify if the antiCaBP antibody.

The PCs in hpc mutants exhibit morphological abnormalities especially of their dendrites, our counts in hpc mutants exhibit morphological abnormalities especially of their dendrites, our counts in hpc mutants exhibit morphological abnormalities especially of their dendrites, our counts in hpc mutants exhibit morphological abnormalities especially of their dendrites, our counts in hpc mutants exhibit morphological abnormalities especially of their dendrites, our counts in hpc mutants exhibit morphological abnormalities especially of their dendrites, our counts in hpc mutants exhibit morphological abnormalities especially of their dendrites, our counts in hpc mutants exhibit morphological abnormalities especially of their dendrites. This resulted in a somewhat reduced litter size. Both treatment groups were similar in gross appearance. Observations of home cage activity, circling behavior, noted that the ethanole exposed progeny were more easily aroused, initiated motor activities more rapidly when aroused, and perseverated more for longer periods of time.

Spontaneous locomotor activity of adult progeny was measured in an automated open field located in a dark room. One male and one female, selected from each litter in the two prenatal treatment conditions, were sequentially placed in the field and left unattended in darkness for thirty minutes during evening hours. Two 15-minute blocks of time, each comprised of three 5-minute trials, were recorded successively for each mouse. Preliminary results indicate that the ethanol exposed mice were no more active than their nutritional controls during the first time block (trials 1-3). The activity of the mice that were prenatally exposed to ethanol did not vary significantly across time. However, pair-fed control mice were consistently more active during the initial 15-minute time block than during the second. Chronic (Randall and Taylor, Teratology, 1979, 19: 305-312), as well as early, (Sulik, Johnson & Webb, Science, 1981, 214: 936-938), prenatal ethanol exposure adversely affected motor activity, in the rat. Sensitivity to ethanol in adult offspring of ethanol-exposed dams was measured in an automated open field (Randall, Recker & Middaugh, Alcohol and Drug Research, 1985, 5: 351-360). Our current work indicates that a late and limited gestational exposure to ethanol also can be associated with behavioral changes in the C57Bl/6J strain.

CELLULAR EVOLUTION, University of Miami, Miami FL
F. Hefti, J. Hartikka, E. Junard*, P. Strange, A. Przylucki, G. Vaughan* and S.W. Fox. Departments of Neurology and Pharmacology, and Institute for Molecular and Cellular Evolution, University of Miami, Miami FL 33101.

Thermal proteins are formed when mixtures of amino acids are heated under simulated geological conditions. These polymers are evolutionary precursors of modern proteins, and are site of aminoo acid sequence (Ivanov and Fortsch, Origins Life 17:35, 1986). Some of these proteins assemble to cell-like structures with properties suggesting prescriptive functions. These findings prompted us to test for effects of thermal protein on neuron cell. We found that these compounds promote survival and fiber elongation of cultured rat brain neurons. Cultures were prepared from dissociated cells of the forebrain of fetal rats (E16). The cells were plated at low density (50,000/cm) in dishes coated with poly-L-lysine and grown in serum-free DMEM or L-15 medium. Cells grow in control medium degenerated within 1-3 days after plating. The addition of some of the thermal proteins resulted in a pronounced prolongation of the survival of the neurons. After 4 days, when control cultures had completely degenerated, treated cultures contained 64% of the originally plated cells. All surviving neurons had various long processes and, using specific neuronal markers, 95% of the cells identified as neurons. Treated cultures degenerated after an apparent delay. Effective concentrations (EC50) of thermal proteins ranged from 3 to 30 μM. All thermal proteins contained aspartic acid and a hydrophobic amino acid. A copolymer poly-(gly,ala,tyr; 6:3:1) synthesized from amino acid carboxyhydrides similar effects effective thermal proteins. The EC50 of this polymer was approx. 0.3 μg/ml. Effective thermal proteins acted in a highly specific manner, since, under the same experimental conditions, various control peptides (e.g., asp, phe), protein (growth factors (NGF, EGF, FGF, insulin) failed to promote the survival of neurons. The effect of thermal proteins was, at least in part, due to their binding to the culture substrate. The effect of thermal proteins was antagonized by heparin, but not by heparan sulfate or chondroitin sulfate.

TROPIC AGENTS I


Mice dams were gavaged on gestation day (GD) 15 with two equal doses of ethanol (total dose, 5.8 g/kg; time, 0 h. and 4 h.). Matched control dams were similarly treated with equivalent volumes of isocaloric dextrose solution and pair-fed for the subsequent 24 h. Live birth rates were the same in both groups. However, the neonatal (postnatal days 0-5) mortality rate was 3 times greater in the ethanol exposed group. This resulted in a somewhat decreased litter size. Both treatment groups were similar in gross appearance. Observations of home cage activity, circling behavior, noted that the ethanol-exposed progeny were more easily aroused, initiated motor activities more rapidly when aroused, and perseverated more for longer periods of time.

Spontaneous locomotor activity of adult progeny was measured in an automated open field located in a dark room. One male and one female, selected from each litter in the two prenatal treatment conditions, were sequentially placed in the field and left unattended in darkness for thirty minutes during evening hours. Two 15-minute blocks of time, each comprised of three 5-minute trials, were recorded successively for each mouse. Preliminary results indicate that the ethanol-exposed mice were no more active than their nutritional controls during the first time block (trials 1-3). The activity of the mice that were prenatally exposed to ethanol did not vary significantly across time. However, pair-fed control mice were consistently more active during the initial 15-minute time block than during the second. Chronic (Randall and Taylor, Teratology, 1979, 19: 305-312), as well as early, (Sulik, Johnson & Webb, Science, 1981, 214: 936-938), prenatal ethanol exposure adversely affected motor activity, in the rat. Sensitivity to ethanol in adult offspring of ethanol-exposed dams was measured in an automated open field (Randall, Recker & Middaugh, Alcohol and Drug Research, 1985, 5: 351-360). Our current work indicates that a late and limited gestational exposure to ethanol also can be associated with behavioral changes in the C57Bl/6J strain.

443.1 NEUROTROPHIC ACTIONS OF THERMAL (ARTIFICIAL) PROTEINS. F. Hefti, J. Hartikka, E. Junard*, P. Strange, A. Przylucki, G. Vaughan* and S.W. Fox. Departments of Neurology and Pharmacology, and Institute for Molecular and Cellular Evolution, University of Miami, Miami FL 33101.

Thermal proteins are formed when mixtures of amino acids are heated under simulated geological conditions. These polymers are evolutionary precursors of modern proteins, and are site of aminoo acid sequence (Ivanov and Fortsch, Origins Life 17:35, 1986). Some of these proteins assemble to cell-like structures with properties suggesting prescriptive functions. These findings prompted us to test for effects of thermal protein on neuron cell. We found that these compounds promote survival and fiber elongation of cultured rat brain neurons. Cultures were prepared from dissociated cells of the forebrain of fetal rats (E16). The cells were plated at low density (50,000/cm) in dishes coated with poly-L-lysine and grown in serum-free DMEM or L-15 medium. Cells grow in control medium degenerated within 1-3 days after plating. The addition of some of the thermal proteins resulted in a pronounced prolongation of the survival of the neurons. After 4 days, when control cultures had completely degenerated, treated cultures contained 64% of the originally plated cells. All surviving neurons had various long processes and, using specific neuronal markers, 95% of the cells identified as neurons. Treated cultures degenerated after an apparent delay. Effective concentrations (EC50) of thermal proteins ranged from 3 to 30 μM. All thermal proteins contained aspartic acid and a hydrophobic amino acid. A copolymer poly-(gly,ala,tyr; 6:3:1) synthesized from amino acid carboxyhydrides similar effects effective thermal proteins. The EC50 of this polymer was approx. 0.3 μg/ml. Effective thermal proteins acted in a highly specific manner, since, under the same experimental conditions, various control peptides (e.g., asp, phe), protein (growth factors (NGF, EGF, FGF, insulin) failed to promote the survival of neurons. The effect of thermal proteins was, at least in part, due to their binding to the culture substrate. The effect of thermal proteins was antagonized by heparin, but not by heparan sulfate or chondroitin sulfate.


Tissue cultures of dissociated neurons have been used to detect neurotrophic factors and study their properties. They provide convenient assays for quantification of polypeptide or polynucleotide activity (Randall, McNeil & Middaugh, Alcohol and Drug Research, 1985, 5: 351-360). Our current work indicates that a late and limited gestational exposure to ethanol also can be associated with behavioral changes in the C57Bl/6J strain.
443.3 THE EFFECT OF POLYAMINES ON MITOCHONDRIAL Ca++ TRANSPORT IN ADULT AND DEVELOPING RAT BRAIN. J. R. Jensen, G. Lynch and M. Baudry. Center for the Neurobiology of Learning and Memory and Dept. of Psychology, Univ. Calif., Irvine, CA 92717.

The polynucleosides, speramine and spermidine, have been shown to increase the rate of Ca uptake by mitochondria from several tissues including brain. As a consequence of the increased rate of transport, mitochondria buffer the extracellular Ca++ concentration ([Ca++]o) to the resting intracellular Ca++ concentration ([Ca++]i). In the present study we investigated further the phenomenon of polynucleotide uptake, and the consequences of the polynucleotide uptake in brain.

In addition, since polyamine levels are high in the postnatal brain, we compared the effect of spermine on mitochondria isolated from adult and developing brain. Spermine not only lowered the steady-state [Ca++]i of isolated mitochondria as previously reported, but also dramatically compressed the rise in [Ca++]i, normally observed with the loading of up to a total of 20 n mole Ca++/mg prot. Analysis of Ca uptake kinetics indicated that the rate of Ca uptake was accelerated by increasing the affinity of mitochondria for Ca++ and by decreasing the cooperativity of uptake. These effects are consistent with an anticompetitive activation of the Ca++ unipporter by polyamines. Moreover, the effects are more pronounced in mitochondria from developing brain and this may be an important source of this growth factor in brain. A short-term allosteric activation of Ca++ uptake by Ca++ was also observed; when a large amount of Ca++ was added in a single load, [Ca++]i would return to values markedly lower than the pre-activation baseline. This effect was absent in mitochondria developing from developing brain and was entirely blocked by spermine, suggesting that spermine can substitute for Ca++ at a allosteric site of the Ca++ unipporter and also that the enhancement of the spermine effect described above is due to the greater cooperative character of Ca++ uptake by adult brain mitochondria.

Since recent evidence supports the view that mitochondria function as Ca++ buffers during periods of increased [Ca++]i, changes in brain polyamine levels may have important effects on the regulation of [Ca++]i. In addition, the enhanced stimulation of mitochondrial Ca++ uptake by spermine observed during the postnatal period, coinciding with higher polyamine concentrations, suggests that [Ca++]i might be regulated at a narrower range of concentrations in developing brain. Furthermore, since a variety of growth factors and hormones produce a rapid induction of ornithine decarboxylase, the rate-limiting enzyme in polyamine biosynthesis, our results suggest an additional possible link between these factors and levels of [Ca++]i.

(Supported by NIH 50-00385 G.L. and MHS-18427 to M.B.)


The polypeptide insulin-like growth factor (IGF-II) peptides within the brain, as well as their cellular identity, have been shown to be important in the regulation of neuronal and adult brain by Northern blot hybridization and the distribution of IGF-I immunoreactivity (IGF-I-ir) in adult brain regions by RIA. The cellular sites of synthesis of IGF-II mRNA in adult rat brain were investigated also in situ hybridization histochemistry (ISH).

Using a rat IGF-II cDNA probe, four IGF-II mRNAs (5.7, 5.4, 1.7 and 1.2 kb) were detected in all regions of neonatal and adult brain. The major differences in IGF-II mRNA abundance were observed in neonatal versus adult brain or in different brain regions. Consistent with this finding were observations of similar levels of IGF-I-ir but different levels of IGF-II-ir in different regions of adult rat brain. The four IGF-II mRNAs were detected also in adult rat pituitary and levels of IGF-II-ir were 4-8-fold higher in adult rat pituitary than in any brain region. The detection of both IGF-I-ir and IGF-II-ir in multiple brain regions and pituitary suggests that these are sites of IGF-I biogenesis. Species of IGF-II mRNA were previously identified in many fetal rat tissues including brain. Recent studies by other groups have shown that these multiple rat IGF-II mRNAs are in part by use of two different promoters and different transcription initiation sites within the IGF-I gene. In the present study we used probes specific for each of these promoters to analyse IGF-II mRNA in neonatal and adult rat brain. Both probes detected multiple IGF-II mRNAs in all regions of neonatal and adult rat brain examined. We found no evidence of regional specific expression of one or other of the two IGF-II promoters in rat brain.

Cellular sites of synthesis of IGF-II mRNAs were analysed in adult rat brain by ISH with synthetic oligonucleotide probes complementary to the polyadenylated 3' end of rat IGF-II cDNA to the 3' end of the rat IGF-I gene. Positive hybridization to cells of the choroid plexus was observed with all three IGF-II oligonucleotides and hybridization occurred throughout choroid plexus. A control oligomer, complementary to rat IGF-I, did not show hybridization to choroid plexus cells. These findings suggest choroid plexus as a primary site of IGF-II mRNA synthesis in adult rat brain. IGF-I has been detected in cerebrospinal fluid by RIA in the present study, but this does not suggest that this derives at least in part from IGF-I synthesis in the choroid plexus.

(Supported by NIH grants NS 14939, 1987 and NS 19964, and March of Dimes grant 1-0121.)
Cortical neurons as previously described (PNAS 83: studies was active, the preparation was tested on cerebellar astrocytes. As little as 1 ng/ml of bFGF was effective, stimulating astrocyte process formation. bFGF also stimulated survival and process outgrowth from cortical neurons (previously described (PMN 85: 2537-7541, 1986). These results clearly demonstrate that different populations of neurons have varied requirements for trophic support.

A second series of experiments examined the binding of bioactive [125I]-labeled bFGF to cultures of highly enriched hippocampal neurons or astrocytes by autoradiography. Label was found associated with neurons above a minimal concentration of 10,000 cpm/ml (about 100 pg/ml) and with astrocytes above 50,000 cpm/ml. The vast majority of a cell's label was in either the cell body or the neurites of the neuron. Even at the lowest concentration tested, the binding of [125I]-labeled bFGF to both neurons and astrocytes was linear for the first 30 minutes of incubation at 37°C. After the first 30 minutes, the binding of [125I]-labeled bFGF to both neurons and astrocytes, suggesting that it too interacted directly with each cell type. If the cells were incubated at 37°C after binding [125I]-labeled bFGF at 4°C, the pattern of label shifted from the cell periphery to the perinuclear region, consistent with the changes expected during internalization of the ligand. It appears that bFGF binds to and is internalized by both hippocampal neurons and astrocytes. The observation that bFGF or bFGF can increase neuronal survival in the absence of astrocytes and the evidence that bFGF binds to neurons in a manner suggesting the existence of a possible astrocyte-axon trophic factor together strengthen the hypothesis that the FGF's are true neurotrophic factors.

Trophic effects of fibroblast growth factor are mediated by direct interactions with hippocampal neurons and astrocytes. Patricia Ann Hallock, Dept. Neurosciences, UCSD, San Diego, CA 92128.

Both basic and acidic fibroblast growth factors (bFGF, aFGF) have been observed to have trophic effects on hippocampal neurons in vitro, increasing neuronal survival and process extension. In addition, both FGF's stimulate astrocytes. We have used two methods to demonstrate that the neurotrophic effects of the FGF's are mediated by astrocyte-receptor interactions with the FGF receptor.

Under these conditions, individual cultures which contained no detectable GFAP+ cells were obtained. Since neuronal survival was still increased by aFGF and bFGF in these glial-free cultures, it is unlikely that either exerts its neurotrophic effects indirectly through astrocytes.

Acidic fibroblast growth factor (aFGF), a heparin-binding polypeptide purified from bovine retina, is not only a potent mitogen for endothelial cells (BR Amore and Klugman, J. Cell Biol. 103: 1363, 1986). We undertook this study to determine how chlormafenicol cells would respond to aFGF and to compare the effects of the two factors on the same cell culture. Disassociated chromaffin cells were grown for 6 days in Medium 199 plus 20% charcoal-stripped fetal calf serum, and then scored for the proportion of cells bearing neurites or labeled on day 6 by a 24 hr exposure to [3H]-thymidine (H-thy). In the absence of added growth factor, less than 3% of the cells exhibited neurites or incorporated H-thy. In the presence of saturating concentrations of 50 ng/ml (300 ng/ml) or 125I-aFGF (125I-aFGF in the presence of 100 ng/ml heparin) roughly 25% of cells grew neurites and a similar number incorporated H-thy. Heparin (100-1000 ng/ml) substantially potentiated the neurite response but depressed the mitotic response. Both NGF and aFGF are mitogenic, but the relative role played by each pathway may vary from one neuronal population to another.

Supported by a research grant from the Muscular Dystrophy Association (P.C.) and NIH grants R01-HD07 (to the Primate Center), ROI-EY0598 (P.A.D.) and ROI-NIT-7283 (J.A.W.).
ACIDIC FIBROBLAST GROWTH FACTOR ENHANCES REGENERATION OF PROCESSES BY POSTNATAL MAMMALIAN RETINAL GANGLION CELLS IN CULTURE. S.A. Linton, J.A. Wagner, R.D. Madison and P.A. D'Amore*. Program in Neuroscience, The Children's Hospital & Dana Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

Acidic and factors represent distinct but homologous gene products. Previously, basic fibroblast growth factor had been shown to promote neuronal survival and process extension in primary cultures of mammalian sensory neurons (Wagner et al., PNAS 83:3012, 1986; Morrison et al., PNAS 83:7537, 1986). In the present study we show that acidic fibroblast growth factor (aFGF) enhances the outgrowth of processes by a postnatal central nervous, the rat retinal ganglion cell (RGC).

RGCs were labeled by retrograde transport of the fluorescent dye granular blue which was applied into the eye of 4- to 10-day old rat pups; two days later the retinas were excised, dissociated, and cultured as previously described (Wagner, Linton, Barnstable & Masland, Science 224:303, 1984). Process extension by RGCs was significantly increased (2.5 fold) in the presence of aFGF after 24 hr in culture, a phenomena RGCs. The addition of heparin (10 μg/ml) to the medium potentiated the action of aFGF on process outgrowth, but heparin itself had no effect. In the presence of heparin, half-maximal process outgrowth occurred at an aFGF concentration of less than 40 pg/ml (2 PM).

Since the centrally projecting processes of RGCs have already been formed in the living animal prior to dissociation, at least a portion of the process outgrowth in culture appears to represent a regenerative phenomenon. The biochemical analysis of the increase in process outgrowth revealed that aFGF with heparin contributed to both neurite initiation and elongation. Masson's trichrome-stained cultures, identified with polyvalent antiserum against glial fibrillary acid protein, was slightly increased in cultures receiving aFGF plus heparin, but this effect was variable, and the glial cells were not in contact with the solitary RGCs that were scored for regeneration of processes. Thus, glial cells probably did not exhibit detectable physical influence on the degree of process outgrowth observed in the solitary RGCs, although a humoral effect cannot be excluded. These results suggest that aFGF has a potent influence on cell processes by a neuron in the mammalian central nervous system. The potentiation of this effect by heparin leads us to speculate that the interaction of aFGF with a heparin-like molecule located in the extracellular matrix (such as heparan sulfate proteoglycan) may produce similar effects in vivo.

Supported by grants EY05474, EY06454, NS00879, and DAMD 17-87-C-7009.


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ACIDIC FIBROBLAST GROWTH FACTOR ENHANCES REGENERATION OF PROCESSES BY POSTNATAL MAMMALIAN RETINAL GANGLION CELLS IN CULTURE. S.A. Linton, J.A. Wagner, R.D. Madison and P.A. D'Amore*. Program in Neuroscience, The Children's Hospital & Dana Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

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Gangliosides have been shown to reduce the damage after a mechanical lesion of the cholinergic or dopaminergic system (Wojcic M. et al. Neurochem. 1977; 33: 505-512; Toffano G. et al. Brain Res. 1983). However, the mechanisms of their action are still unknown, and they could either foster neuronal sprouting or prevent cell death in the vicinity of the lesioned area. In order to study ganglioside effects on the 5-hydroxytryptamine (5-HT) system we used two types of lesion of the 5-HT afferents to rat hippocampus. The first lesion consists in cutting unilaterally the fimbria-fornix using a razor blade (Moroni F. et al. Brain Res., 150:333, 1978). Seven days after this procedure the 5-HT and the 5-hydroxyindole acetic acid (5-HIAA) content decreased by 78% and by 65% respectively in the dorsal and by 50% and 40% respectively in the ventral hippocampus. However, 60 days later a partial recovery, possibly due to a collateral sprouting, does occur. The second lesion consists in an electrolytic destruction of a mesencephalic area at the following coordinates: AP -4.9. L +1. H +7.4 (Konig-Klippel 1963). This lesion decreases the 5-HT and the 5-HIAA content by 30% and by 20% respectively in the total hippocampus. A treatment with GM1 ganglioside (30 mg/kg/day/2 weeks) failed to prevent the sprouting of 5-HT neurons which were present in fimbria-fornix rats 60 days after the surgery. In rats having an electrolytic destruction of the mesencephalic area the fimbria-fornix pathways are severed, but it is able to reduce the damage of an electrolytic mesencephalic lesion when the fimbria-fornix pathways are severed, but it is able to reduce the damage of an electrolytic mesencephalic lesion possibly preventing the death of neurons in the vicinity of the lesioned area.

443.16 ADMINISTRATION OF GM1 GANGLIOSIDE RESTORES DOPAMINE CONTENT IN THE STRIATUM OF MPTP-TREATED MICE. M. BADALOCONSTANTINO AND B. FASSO. Department of Pharmacology, The Ohio State University College of Medicine, Columbus, Ohio USA 43210.

There is experimental evidence documenting that administration of GM1 ganglioside stimulates the recovery of dopaminergic nigrostriatal neurons after different types of lesions. Apparently, GM1-ganglioside administration has a neuroprotective effect on the primary lesion and on the secondary retrograde degeneration following nerve terminals lesions. It may affect the activity of neurotrophic and neurogenic factors and their expression at the level of the lesion which influence regeneration. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a neurotoxin that produces parkinsonism in humans and monkeys and degeneration of nigrostriatal neurons in various species. We now report that the administration of GM1 restores the dopamine (DA) content in the striatum of MPTP-treated mice. Mice were treated with MPTP, 20 mg/kg, i.p., for 7 days. This treatment resulted in a loss of neurons in substantia nigra and about a 60% decrease of DA and DOPAC in the striatum. On day 8, GM1, 30 mg/kg, i.p., or the synthetic ganglioside analogue K-2, 20 mg/kg, i.p., was administrated daily for 2 days. Drug treated control groups were evaluated as well. Treating mice with GM1 or K-2 alone had no significant effect on the striatal DA or DOPAC content. Both GM1 and K-2 restored the DA content in striatum of MPTP-treated mice. This effect was dose- and time-dependent. Maximum effect was observed after about 3 weeks of treatment with 30 mg/kg, i.p., of GM1. GM1 increased the DOPAC content in the striatum of the MPTP-treated mice over control values, suggesting stimulation of DA synthesis. Indeed, when the DA synthesis was studied directly, we found that GM1 administration caused a stimulation above control DA turnover values in the striatum of MPTP-treated mice.


Alzheimer's disease includes pathological effects on cholinergic forebrain neurons. In an attempt to study the cholinergic aspect of this disabling disorder, animal models of the disease have been devised. These experiments generally involve lesioning of specific cerebral or subcortical areas of CNS cholinergic neurons. Such models produce the characteristic decrease in choline acetyltransferase (ChAT) activity and cell loss seen in the human condition. Whether this degeneration occurs retrograde to the active fibre or anterograde to the active axonal insult remains to be determined. In the present set of experiments animals were unilaterally decorticated by disrupting the blood vessels which supply the cortex. This devascularizing lesion produces a retrograde-type degeneration of the cholinergic neurons originating from the nucleus basalis magnocellularis (NBM), as previously described (Sofroniew et al. Brain Res. 289:370-374, 1983; Stephens et al. J. Neurochem. 45:1021-1025, 1985). Forty-five subjects (male Wistar rats: 350-550g) were used in the study: 25 control and 20 lesioned subjects. Gtx of each group were infused with the ganglioside GM1 (5mg/kg/day: 14 days) using minipumps (ALZET) connected to chronically implanted intracerebroventricular cannulae. One month following the initial lesion, several subcortical and cortical brain areas were assayed for ChAT activity. In agreement with previous data (Sofroniew et al Brain Res. 376:373-377, 1986) the cortical lesions produced a 30-50% decrease in ChAT activity in the ipsilateral NBM and this effect was prevented by administration of GM1. Prior to the surgical manipulations and immediately before the subjects were sacrificed, each animal was tested in a variety of behavioral paradigms (sensorimotor testing, pain sensitivity, locomotor activity and memory testing; passive avoidance and Morris water maze). Correlations between the biochemical and behavioral data will be reported. Supported by the Canadian MRC and Fidia spa.

443.18 GANGLIOSIDE-ENHANCED RECOVERY OF SPATIAL ALTERNATION PERFORMANCE AFTER UNILATERAL ENTORHINAL CORTEX LESIONS IN MICE. J.B. RAMIREZ1, B. FASSO1, D. ZEMPEL1, P. WRIGHT1, AND S. KARPIAK1. (SPORs. R. KAPLAN). Department of Pharmacology, University of Miami, Med. School, Louisville, KY 40208. Supported by NIH grant 5R01 NS25979 (B.F.), and the National Institute of Mental Health (B.F.).

Exogenous gangliosides have been shown to improve behavioral recovery after brain damage. In our previous studies, rats treated with mixed gangliosides throughout 3 weeks of postoperative behavioral testing showed better retention of a learned alternation task than saline-treated rats after unilateral entorhinal (Kaplan, 1983; Ramirez et al., 1987). The present study was performed to determine whether short-term ganglioside treatments would facilitate recovery of alternation behavior after unilateral entorhinal cortex lesions. Gangliosides were administered during the early postlesion interval; i.e., the day preceding surgery and the next 6 days. This interval was just prior to the onset of crossed tem- porodependent sprouting, which has been implicated in the recovery of alternation (Loesche & Steward, 1977).

Male Sprague-Dawley rats sustained unilateral entorhinal lesions or craniotomies and were tested for retention of a preoperatively acquired alternation task (1 maze). Ganglioside (50 mg/kg) and saline (0.9%) i.e., injections were administered for 7 consecutive days beginning on the day before surgery. The rats were tested postoperatively until the attained criterion performance (3 consecutive days of 80% alternation), or for a maximum of 3 weeks. Although the extent of damage was comparable between the two lesion groups, the ganglioside-treated group performed better than the saline-treated rats. The former required fewer postlesion days to reach criterion performance. Thus, a short-term regimen of ganglioside treatment is effective in promoting behavioral recovery after unilateral entorhinal lesions (e.g., if i.e., injections were administered for 7 consecutive days beginning on the day before surgery). However, it cannot be concluded that exogenous gangliosides alter acute and long-term postoperative events (e.g., edema, membrane failure, sprouting). These events may contribute to the enhanced recovery observed in the present study. The relative degree to which these events contribute remains to be determined. Supported by NINH grant R4273S (B.F.).
443.19 GM1 GANGLIOSIDES ARE INEFFECTIVE IN REDUCING AMPHETAMINE-INDUCED ROTATIONAL ASYMMETRY, BUT PROTECT RATS FROM APHAGIA AND ADIPSIA FOLLOWING COMPLETE NIGROSTRIATAL HEMISECTIONS. G.L. Dunbar, C. Faggiontoni, and D.O. Stein. Brain Res. Lab., Clark University, Worcester, MA 01610; now at Dep. of Psychology, Central Michigan University, Mt. Pleasant, MI 48859.

Since the effect was first reported by Toffano et al. (1), studies have consistently shown that treatments with GM1 gangliosides attenuated the loss of striatal tyrosine hydroxylase (TH) levels and reduce rotational asymmetries in rats with partial hemisections of the nigrostriatal pathway. However, subsequent work (2) shows that after extensive hemisections of the nigrostriatal pathway, the effect of GM1 on striatal TH levels disappears. This study investigates whether extensive hemisections also eliminate the GM1 effect of reducing rotational asymmetry. Since these treatments often cause aphagia and adipsia in rats, we also measured whether GM1 treatments could counteract the severe weight loss observed after this injury.

Seventeen, male albino rats were given complete hemisections of the nigrostriatal pathway, following the parameters used by Toffano (2). One group (n=9) received daily IP injections (200ug/kg) of GM1 gangliosides (Fidia Research Laboratories), beginning immediately after surgery and continuing for two weeks. Another group (n=8) received the same regimen of IP injections of saline. Amphetamine-induced ipsiversive rotations were assessed at postoperative days 2, 7, and 14. Daily body weights were taken for both groups.

Results revealed that gangliosides had no effect on the number of ipsiversive rotations during any of the testing days, but significantly reduced the amount of weight loss (3). It is not known how gangliosides protect rats from aphagia and adipsia following this lesion, but the protection observed suggests that it probably involves mechanisms other than dopaminergic innervation of the striatum by nigrostriatal fibers.


This study was supported by a grant from Fidia Research Laboratories Albano Terme, Italy.

443.20 GANGLIOSIDES GM1 AND AGF2 PROMOTE RECOVERY OF WORKING MEMORY FOLLOWING COLCHICINE-INDUCED GRANULE CELL LOSS IN RAT HIPPOCAMPUS. D.E. Farneth and T.J. Walsh. Dept. of Psychology, Rutgers University, New Brunswick, NJ.

GM1 ganglioside promotes recovery of function following various forms of neural insult. This compound has been shown to attenuate the cognitive and motor impairments following occlusion (COL) induced granule cell loss in rat hippocampus (Farneth et al, Soc. Neurosci. Abstr. 1987). These experiments suggest the ability of GM1 as well as its internal ester, AGF2, to attenuate the cognitive impairments resulting from the destruction of this neural population.

Rats were trained on a multiple component T-maze task (Cherinah et al. Br. Res. 1967). Animals were placed into one of two start/ goal boxes located on either arm of the maze. They were trained to run down the arm of the maze and return to the same start/goal box for food reward (i.e., a non-match to sample task). The location of the start box was randomly alternated on each trial. The different aspects of this task may be characterized as: 1) a reference memory component which requires discrimination of changing stimulus features (i.e., the start location) to perform accurately. Following acquisition of the task, rats were injected (IP) with either GM1 (30 mg/kg), AGF2 (10 mg/kg) or the saline vehicle (SAL) for three days prior to surgery and for 14 days thereafter. COL 0.5 mg/kg) or SAL was histionically injected into the dentate gyrus at two rostral-caudal locations. Both COL and SAL-treated animals exhibited a transient impairment on the reference memory component which recovered within 15 trials. GM1 and AGF2 did not alter the rate at which reference memory recovered. While control animals required 55 trials to reach a criterion of 70% accuracy on the working memory component, rats injected with COL (COL-SAL) were markedly impaired on the working memory component and required 110 trials to reach the same level of performance. Rats treated with GM1 (COL-GM1) required 95 trials and rats treated with AGF2 needed 70 trials to reach criterion. Thus, GM1 and, in particular, AGF2 enhanced the rate of recovery of normal working memory processes.

Histological analysis revealed that COL produced a 55% decrease in the thickness of both the superior and inferior borders of the dentate gyrus due to the marked loss of granule cells. This was accompanied by a slight decrease in hippocampal volume, mild ventricular dilatation, and a small (10%) decrease in the thickness of the CA1 pyramidal cell field. Neither GM1 or AGF2 affected these secondary changes resulting from cell loss.

The beneficial effects of these neuroactive agents are probably not related to a decrease in the impact of COL on its population of target neurons (granule cells) but rather could represent a reduction in secondary changes following cell loss, or the facilitation of behavioral and/or neuronal reorganization that promotes functional recovery.

These data further support the contention that certain gangliosides can facilitate recovery of function following destruction of specific populations of cells, a common finding in neuropathological diseases.

Supported by grants from Volkswagenwerk Foundation (Un 34/11-1) and Volkswagenwerk Foundation.
444.3 NEUROTROPHIC FACTORS IN ADRENAL MEDULLARY CELLS: DEVELOPMENT, RELEASE AND MOLECULAR CHARACTERISTICS. K. Unsticker, D. Blottner*, D. Geheber, F. Stibbe, R. Wernemeyer, R. Fischer-Colbrie*, R. Winkler, and P. G. Gehrke*. Dept. of Anatomy and Cell Biology, University of Marburg, F.R.G., Pharmacology, University of Innsbruck, Austria, and Biochemistry, University of Zurich, Switzerland.

Target-dependent neuronal death is a basic phenomenon in nervous system development. With regard to neuron-mediator interactions it would imply that neurons may store and secrete neurotrophic factors (NTFs) that support survival and differentiation of their presynaptic counterparts. We have previously shown that proteins stored in chromaffin vesicles of bovine adrenal medullary cells promote in vitro survival of embryonic spinal cord, sensory and sympathetic neurons innervating the adrenal medulla in situ (Soc. Neurosci. Abstr. 11/2, p. 1083, 1985). We report here on development, ESTASE and further molecular characteristics of these NTFs. Development: High speed supernatants of homogenates from carefully dissected rat adrenal medulla (postnatal day P7) and P9) contain NTF activity for embryonic chick ciliary (day 8; CG8), dorsal root (DRG8, DRG10) and lumbar sympathetic (SG11) ganglion neurons from P8 onwards, i.e. shortly after the onset of the functional preganglionic innervation of the medulla at P5 (ED8: 10 μg protein/ml). Homogenates of adrenal cortex had weak activity for SG1, but none for the other neurons tested. Release: NTF activities were released from isolated bovine chromaffin cells upon cholinergic stimulation by a Ca2+-sensitive mechanism. Molecular characteristics: NTF activity residing in isolated bovine chromaffin granules was not blocked by antibodies to NGF and chromogranins A, B, C. After separation on HPLC molecular sieve columns an activity addressing CG8 neurons eluted at Mw = 22 Kd. Activities for CG8 and DRG8 neurons could also be enriched on DEAE Sephadex anion exchange and high-performance affinity columns. Preliminary data from 'cell blots' (Carnow et al., J. Neurosci. 5, 1985) revealed CGB neurotrophic activity on 4 distinct bands with Mr = 24, 34, 55 and 95 Kd. 24 Kd was the molecular weight of CNTF isolated from rat sciatic nerve (Manthorpe et al., Brain Res. 367, 282, 1986). These results suggest (i) tissue and target neuron specificity, (ii) presence of a CNTF-like protein and (iii) differences in the regulation of neuronal death we investigated the effect of BTX on the number of CGR+ neurons in individual pools. Many CGR+ cells clustered to form islands of CGR+ neurons in the feline spinal cord. In contrast, CGR+ cells were observed in the femoro-tibialis, ilio-fibularis, illofibularis and iliopsoas. To our knowledge, this is the first demonstration of biochemical differences between motoneurons projecting to different targets.

444.4 CILIARY NEUROTROPIC FACTOR IN EYE TISSUES OF AVIAN EMBRYOS TREATED WITH ALPHA-BUNGAROTOXIN H.J.L. Frere*, S.D. Meriney, and D.A. Brown. MRC Developmental Neurobiology Prog., Dept. Zoology, University College London, London WC1E.

Calcitonin gene-related peptide (CGRP)-like immunoreactivity has been observed in a subpopulation of motoneurons in chick spinal cord from day 6 (26) onwards, indicating the presence of CGRP-like immunoreactivity in motoneurons. In the chick, the ciliary subpopulation of CG neurons are like motoneurons in that they are cholinergic and project to striated muscle. Firstly, immunohistochemistry for CGRP was performed on the developing ciliary and iris muscle, the two targets for CGR+ neurons. CGRP was present in both of these sites during a restricted phase of development. In the ciliary muscle staining was first seen in myoblasts at E9; peak staining intensity was reached at E11 and E13 and staining was absent by E18; in the iris, staining was seen at E11 and E13. Neuronal death in the chick occurs between ER-E14 and so these findings are consistent with a potential role for CGRP as a trophic factor during this period. Secondly, a 48 hour survival assay for ER-E9 chick CG neurons has been established in vitro. The dissociated cells are grown in a high potassium medium on a matrix produced by Schwann cells. We have confirmed that saline extracts of ER-E10 skeletal muscle support neuronal survival, whilst synthetic VIP does not. We are currently using affinity-purified antibodies to establish the role of VIP in supporting survival of these CG neurons.

444.5 A PUTATIVE TROPHIC FACTOR FOR CILIARY GANGLION NEURONS IN SKELLETAL MUSCLE. C.G. Mudge and A.W. Mudge*. (MRC: J.G.R. Jeffery). MRC Developmental Neurobiology Prog., Dept. Zoology, University College London, London WC1E.

Previous work (A.W. Mudge and H.V. New [1986] Soc.Neurosci. Abstr. 300, 1) described a putative motoneuron trophic factor, intrasomatic VIP (IMF), which is present in developing chick skeletal muscle. IMF is recognized by some antibodies directed to the amino terminal end of vasoactive intestinal peptide (VIP). IMF extracts were shown to contain immunoreactive VIP. Further, rabbit immunoglobulins are filtrate containing two major peaks of molecular mass 20KD and 10KD. It is present in all regions of the chick embryo. In the present study we have investigated the possibility that IMF might be a trophic factor for ciliary ganglion (CG) neurons. In the chick, the ciliary subpopulation of CG neurons are like motoneurons in that they are cholinergic and project to striated muscle. Firstly, immunohistochemistry for IMF was performed on the developing ciliary and iris muscle, the two targets for CGR+ neurons. IMF was present in both of these sites during a restricted phase of development. In the ciliary muscle staining was first seen in myoblasts at E9; peak staining intensity was reached at E11 and E13 and staining was absent by E18; in the iris, staining was seen at E11 and E13. Neuronal death in the chick occurs between ER-E14 and so these findings are consistent with a potential role for IMF as a trophic factor during this period. Secondly, a 48 hour survival assay for ER-E9 chick CG neurons has been established in vitro. The dissociated cells are grown in a high potassium medium on a matrix produced by Schwann cells. We have confirmed that saline extracts of ER-E10 skeletal muscle support neuronal survival, whilst synthetic VIP does not. We are currently using affinity-purified antibodies to establish the role of VIP in supporting survival of these CG neurons.


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444.7 A SUBTRACTIVE-SCREENING FACTOR FROM MUSCLE THAT PROMOTES NEURITE OUTGROWTH FROM NEURONS OF THE CENTRAL AND PERIPHERAL NERVOUS SYSTEMS. 

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Spinal motor neurons depend upon muscle-derived trophic factors for their development and survival. Some circumstantial evidence suggested to us that the regulatory subunit of 3',5'-cyclic-adenosine monophosphate-dependent protein kinase (cAMPPK) type II might be involved in neurite outgrowth from spinal neurons (Gomez, M., Nature, 307: 549; Held, J. R., et al., J. Neurosci., 3: 2045, 1983). In the present study, we tested a commercial preparation of cAMPPK for its neurite-promoting activity on chicken embryonic spinal neurons in culture. Commercial cAMPPK type II from skeletal and cardiac muscles elicited a significant neurite outgrowth from cultured neurons when the enzyme preparation was bound to polylysine-coated substrata; type I cAMPPK from skeletal muscle was ineffective. Neither cAMPPK type I nor type II had a significant effect on the survival of spinal neurons in culture. Type II cAMPPK also stimulated neurite outgrowth from chicken cerebral hemisphere neurons, dorsal root ganglion cells, ciliary ganglion neurons and rat sympathetic ganglion neurons in culture. However, as neither the purified regulatory nor catalytic subunits of cAMPPK type II had an effect on neurite outgrowth per se from cultured neurons, and as neurite-promoting activity was not correlated with 3H-cAMP binding nor cAMP-dependent kinase activity, we reasoned that neurite-promoting activity resided in a contaminant of the preparation. The neurite-promoting protein was partially purified from commercial cAMPPK type II by gel filtration on Sephadex 2-200 followed by ion-exchange chromatography on Mono-Q. SDS gel electrophoresis of the active protein fraction revealed a major protein band (MW 50 kd) and several minor bands (e.g., MW 200 kd, 45 kd etc.). Western blot analysis and immunoprecipitation revealed that the neurite-promoting protein was distinct from laminin, nerve growth factor, neural cell adhesion molecule. Further evidence that neurite-promoting protein was not retained on a heparin affinity column nor destroyed by heparinase. Our results demonstrate that a factor which type II cAMPPK cAMP-dependent protein kinase of striated muscle stimulates neurite outgrowth from neurons of the central and peripheral nervous systems. Supported by the NIH (NS 15013-05R and NS 20490-05R).

444.8 PURIFICATION OF A NEUROTROPHIC FACTOR FROM PERIPHERAL NERVES P.M. Richardson, M. Altarese and R.J. Rimpelle. 

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A purification scheme is reported for a molecule that supports chick ciliary neurons in tissue culture and is relatively concentrated in peripheral nervous tissue (Chenel et al, Neurosci. Lett. 14, 91 (1987); Barbin et al., J. Neurochem. 43, 1468 (1984); Collins, Dev. Biol. 109, 255 (1985)). Such peripheral sciatic nerves are pulverized in a buffer with protease inhibitors and defatted with petroleum ether. The homogenate is then loaded on preparative and semi-preparative C18-silica columns, bioactivity eluting from the latter column with 55-60% acetonitrile in 0.1% trifluoroacetic acid. Further purification involves the addition of 90-95% specific activity on simple exposure to acetonitrile, enough bioactivity remaining after reverse-phase HPLC to monitor further purification. The next step in purification involves an ion-exchange column (Mono-Q) and elution with a gradient of ammonium acetate in water. Finally bioactive fractions are loaded on a C4 silica column and eluted again with acetonitrile-trifluoroacetic acid. The yield from these procedures is in the order of 1 ng/ml of a product that is bioactive on chick ciliary neuron survival at concentrations below 1 ng/ml even after denaturation. The apparent molecular weight of the neurotrophic activity is approximately 13,000 on gel-exclusion HPLC. Because of the denaturation, the purification protocol is appropriate for biochemical rather than physiological studies.


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Neurotrophic factors are generally assumed to be soluble because of the difficulty in recognizing specific properties which allow for their unambiguous identification. However, the activity is likely that many CNS neurons also require specific trophic factor support, their requirements have not been assessed because of the difficulty in recognizing specific types of CNS neurons in heterogeneous cultures. Recently developed cultures of chick embryo retinal neurons and photoreceptors have overcome this drawback. Cells in these cultures express many differentiated properties which allow for their unambiguous identification by morphological, immunocytochemical and autoradiographic techniques (rev. Adler, R. Prog. Ret. Res. 6:1, 1985). A possible source of putative trophic factors for photoreceptors is the interphotoreceptor matrix (IPM), a mixture of proteoglycans and glycoproteins localized between photoreceptors and the retinal pigmented epithelium. IPM preparations (isolated as described by A. Adler, R. Prog. Ret. Res. 6:1, 1985) were found to promote the survival of cultured photoreceptors by up to 400%, without affecting the survival of the THIP photoreceptor-specific response was not mimicked either by known growth factors, such as NGF (gift of M. Meisner), or by Interphotoreceptor Retinoid-Binding Protein (gift of G. Chader), the best characterized IPM component. IPM preparations can exert their photoreceptor-survival promoting effect either when added to the medium, or when substrate-bound. The activity is nondialysable, heat-labile and inactivated by pH 5.0 and 10.0. Activity can be recovered from Sephagly C-4 silica by gel filtration column chromatography and from Dae-Sephael from which it can be eluted with 0.25 M HC1. This evidence indicates that an activity that specifically promotes photoreceptor survival in vitro is associated with macromolecular IPM. Further purification of the activity and generation of antibodies will be needed in order to establish whether this activity regulates photoreceptor survival in vivo.

444.10 CHARACTERIZATION OF A BPHEMATOPOIETIC MATRIX PREPARATION THAT PROMOTES PHOTORECEPTOR SURVIVAL IN RETINAL MONOLAYER CULTURES. J. R. Lindsey, A. T. Hewitt and R. Adler.

Wynn Center, Wilmer Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The survival of many peripheral neurons is specifically regulated by the retinal pigmented epithelium. IPM preparations (isolated as described by A. Adler, R. Prog. Ret. Res. 6:1, 1985) were found to promote the survival of cultured photoreceptors by up to 400%, without affecting the survival of the THIP photoreceptor-specific response was not mimicked either by known growth factors, such as NGF (gift of M. Meisner), or by Interphotoreceptor Retinoid-Binding Protein (gift of G. Chader), the best characterized IPM component. IPM preparations can exert their photoreceptor-survival promoting effect either when added to the medium, or when substrate-bound. The activity is nondialysable, heat-labile and inactivated by pH 5.0 and 10.0. Activity can be recovered from Sephagly C-4 silica by gel filtration column chromatography and from Dae-Sephael from which it can be eluted with 0.25 M HC1. This evidence indicates that an activity that specifically promotes photoreceptor survival in vitro is associated with macromolecular IPM. Further purification of the activity and generation of antibodies will be needed in order to establish whether this activity regulates photoreceptor survival in vivo.

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We have previously reported that bovine striatal extracts are capable of increasing the survival and maturation of fetal mouse mesencephalic neurons maintained in serum-free culture conditions. (Dal Toso et al., Soc. Neurosci. Abstr., Vol.12, Part 2, p.1100, 1986). This biological activity is associated with a fraction whose main component is a basic protein of approximately 14 kDa. Since several defined growth factors have been reported to possess similar effects on cultured CNS neurons, we evaluated the chemical characteristics, as well as the biological activity of SDNF in comparison to Nerve Growth Factor (NGF) and basic Fibroblast Growth Factor (bFGF). SDNF, like NGF, is able to promote neurite regeneration in NGF-primed PC12 cells; however, unlike SDNF, NGF is totally without effect on the mesencephalic neurons. In contrast, both SDNF and bFGF display similar activities toward the mesencephalic neurons. When applied to a hirudin clot, the 14 kDa protein of the most pure SDNF fraction shows a different elution profile with respect to bFGF. Experiments are currently in progress to further compare SDNF to bFGF, utilizing chemical and immunological techniques, as well as the biological activities addressed in a variety of neuronal and non-neuronal cell types.
THE LAMININ BINDING PROPERTIES OF RAT PC12 CELLS.

Na+ channels were recorded. Furthermore the cultures were characterized by light microscopy, transmission and electron microscopy as well as by immunocytochemical methods. The proportion of glial acidic protein (GFAP) positive cells gradually decreased from an initial 80% to about 10-20%, while the neuronal cells increased accordingly. Thus 3 week old cultures contained essentially only neurons. Autoradiography with 3H-thymidine also showed neuronal cell division.

Using the patch clamp technique, voltage dependent Na+ channels were recorded. Furthermore these cultures produced neuropeptides such as substance P and substance K, somatostatin and express cholinergic properties as indicated by choline acetyltransferase activity.

In conclusion, we have established the conditions to grow neuronal cells from human fetal brain in a chemically defined medium. The use of frozen material allowed pooling of tissues of comparable gestational age and to carry out relatively large scale experiments. Such cultures may serve as a useful tool to address a variety of questions on human brain differentiation including neuronal development, the actions of neurotrophic factors as well as neurotoxicity screening.

THE LAMININ BINDING PROPERTIES OF RAT PC12 CELLS.

The basement membrane protein laminin has been shown to potentiate the survival and neurite outgrowth of target neurons of both NGF and NGF-derived neurotrophic factors (D. Edgar et al., EMBO J., 3:1463, 1984; R.M. Lindsay et al., Dev. Biol., 122:319, 1985). It has been found that a specific domain of the laminin molecule including the end of the long arm and the heparin-binding globule is associated with its effects on chick sympathetic neurons (D. Edgar et al., EMBO J., 3:1463, 1984). This laminin fragment has also been shown to selectively increase the levels of those enzymes specifically involved in the catecholamine biosynthetic pathway in calf adrenal chromaffin cells (A. Acheson et al., J. Cell Biol., 102:151, 1986). The aim of the present study was to document the effects of laminin fragments on PC12 cells, prior to the characterisation of laminin-binding proteins from cell membrane preparations.

Laminin and laminin fragments prepared from EHS sarcoma (R. Timpl et al., J. Biol. Chem., 254:9933, 1979) were radioiodinated, and ligand-binding studies to intact, suspended cells at 0°C performed. Subsequent Scatchard analysis revealed a single class of high affinity receptors with a K, of 3x10⁵ M^-1. Of the 6000 binding sites per cell, almost all bound the end of the long arm. Whole laminin binding could be blocked with fragments corresponding to the long arm of the molecule including the heparin-binding domain, but not by other fragments including those derived from the short arm. The active fragment was also able to reproduce the potentiating effects of whole laminin on PC12 cell neurite outgrowth, enzyme induction, and response to NGF. It is concluded that, as is the case for neurons, the end of the long arm mediates the binding of laminin to PC12 membranes.

PURIFICATION AND CHARACTERIZATION OF NEURONAL PRECURSOR CELLS FROM NEONATAL RAT ADRENAL GLANDS.

Adrenal medullary cells and sympathetic neurons are neural crest derivatives that become committed to express endocrine-like (chromaffin cells) or neuronal-like (sympathetic neurons) phenotype depending upon the environment in which they reside and the repertoire of trophic molecules to which they are exposed. A subpopulation of chromaffin cells retain the ability to express neuronal phenotype in response to Nerve Growth Factor (NGF).

Since neuronal cells are post-mitotic, the availability of neuronal precursor cells and the understanding of the environmental factors that control expression of neuronal phenotype has important implication for successful neuronal regeneration and functional recovery in the adult central and peripheral nervous system. Adrenal medullary cells obtained from embryonic, neonatal or adult animals and grown in culture have been shown to express neuronal-like morphology when grown in the presence of NGF. A monoclonal antibody that recognizes NGF receptors (NGFR) (IgG-192, gift from Dr. Eugene Johnson) was used to label the potential NGF responsive cells. A subpopulation of NGF positive cells was identified. Both the number of stained cells and the intensity of staining increased following exposure to NGF. In order to purify the subpopulation of NGF bearing cells, adrenal glands were removed from postnatal day 7 rats, the adrenal medulla was separated from the adrenal cortex under a dissecting microscope, enzymatically dissociated and pre-incubated with NGF (50 ng/ml) for 48 hours at 37°C in humidified chamber with 5% CO₂ in air. Following preincubation, the cells were labeled with the anti-NGF monoclonal antibody and visualized by a fluorescence detection system consisting of biotinylated horse anti-mouse IgG and avidin conjugated fluorescein. Cells were sorted based on fluorescence intensity and forward light scatter. Positively fluorescent cells as well as non-fluorescent cells were collected separately under viable and aseptic conditions and plated on collagen coated microwell plates, or frozen in liquid N₂ for further studies. Approximately 3-10% of the sorted cells were NGFR positive and better than 90% of the sorted NGF positive cells expressed neuronal morphology when grown in the presence of NGF. Further characterization indicates that these cells contain tyrosine hydroxylase (TH) as determined by immunocytochemistry using anti-TH antibodies and by hybridization studies using TH mRNA as the probe. In summary, we have purified a subpopulation of neuronal precursor cells from adrenal medulla that are NGF responsive and resemble sympathetic neurons in their neurotransmitter choice.
In vitro studies on the role of activity in the expression of a synaptic vesicle protein in rat superior cervical ganglion cells. G. Greif and K. R. Linderman. Soc. Neurosci. abstr. 12: 1109, 1986). However, weight reductions and other effects of synaptic vesicles. Neonatal deafferentation of the superior cervical ganglion (SCG) produces a reduction in SV levels, a result which seems to be independent of any toxic side effects like the usefulness and clarity of these experiments. Therefore, the neurophysiological system was adapted to investigate the role of activity in the regulation of SV expression. SV levels were quantified by radioimmunoassay.

In SCG explants, cultured in defined medium (Kessler et al., Neurosci. 9: 309, 1983) SV levels doubled in the first two days and gradually declined over two weeks, but never fell below levels expressed in two day old SCG. However, total protein content of the ganglia also declined rapidly in the first two days in culture, so that SV levels standardized for ganglion size rose approximately 60% and were equal to initial levels after 14 days in culture. These results may reflect non-neuronal cell death since the medium used does not support survival of these cells. The results thus suggest that removal of culture prevents normal postnatal increase in SV in the SCG.

Activity of cultured SCG was modified by the addition of the depolarizing agent, veratridine (VER, 10^{-7} M), potassium (30mM), and/or low calcium (1mM) to the medium. High activity cultures (+VER) showed significant increases (>150%) after 24 hours of treatment and levels remained high with sustained treatment for 14 days. This induction of SV was reversibly decreased by concurrent administration of TTX with VER. High K+ medium also caused smaller increases in SV levels; increases were not completely reversible by TTX. These in vitro studies support the in vivo findings that activity plays a role in the regulation of expression of SV in the SCG during postnatal development. Other transsynaptic factors involving target interactions or other factors may also be important.

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### Synergistic Effects of Muscle and Central Glial-Derived Trophic Factors on the Survival of Embryonic Chick Motor Neurons in Culture

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Identified and isolated spinal motor neurons from 6-day-old chick embryos are unable to survive in culture unless provided with a soluble trophic factor from embryonic muscle cells (A.L. Calof and L.F. Reichardt, Dev. Biol. 106:194-210; U. Dohrmann et al., Dev. Biol. 118: 209-221). However, some motor neurons, identified by retrograde labeling with rhodamine, will survive in mixed spinal cord cultures in the absence of muscle extract. This survival promoting activity is a protein secreted by glial cells from the central nervous system (CNS), and its production is enhanced by factors known to stimulate the proliferation of glial cells such as the glial growth factor GGF (G.E. Lamke and J.P. Brooks, J. Neurosci. 1:75-81).

At optimal concentrations, the CNS derived trophic activity can support the same number of cultured motor neurons as muscle extract. However, the two trophic activities interact in a synergistic manner: A) The CNS glial derived activity potentiates that of muscle to decrease the number of neurons lost, B) Motor neuron survival15 seen in the presence of both factors is more than the sum of their individual activities.

A survival promoting activity similar to that from CNS glial cell conditioned medium is also detected in extracts from both embryonic and adult spinal cord and brain which suggests that the CNS derived trophic activity might play a role in the maintenance of adult motor neurons.

As a series of defined growth and survival factors present in the CNS, brain derived growth factor, acidic and basic fibroblast growth factor have no effect on motor neuron survival, then we conclude that the molecule responsible for the motor neuron survival promoting activity of the CNS is probably a growth factor.

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### Noradrenergic Character of Chick Sympathetic Neurons Is Enhanced by Non-Neuronal Cells

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Noradrenergic character of sympathetic neurons (SN) derived from peripheral sympathetic ganglia of 10-12-day-old chick embryos and maintained in vitro under serum-free conditions was examined by measuring norepinephrine (NE) content and ³H-NE uptake. SN were cultured in a serum-containing or serum-free medium as described before (Wakade et al., Devl. Cell Res. 40, 71, 1983). SN supported in culture in a serum-free or serum-containing medium showed almost equal equilibrium ³H-NE uptake (3.4 x 10⁵ CPM/dish).

A significant uptake (181 x 10⁵ CPM/dish) was observed when SN were co-cultured with chick heart or liver cells in the absence of NGF for 3 days. Addition of NGF to these co-cultures caused a marked facilitation of ³H-NE uptake (373 x 10⁵ CPM/dish).

Uptake of ³H-NE was facilitated when SN were co-cultured with chick skeletal muscle cells; however, the facilitation was evident only in the presence of NGF. When the SN were co-cultured with sensory neurons there was again a marked facilitation of uptake in the absence as well as the presence of NGF. The facilitatory effects of heart or liver cells on the uptake of ³H-NE by SN was present for 6 days in culture. SN in a medium conditioned by either of these target cells also resulted in a facilitation of the uptake. SN initially selected in culture by NGF in regular medium and then exposed to heart-conditioned medium for 3 days exhibited an increase in ³H-NE uptake. This was approximately 5- to 6-fold increase in NE contents of SN co-cultured with non-neuronal cells, and about 2-fold increase when SN were grown in a medium conditioned by the non-neuronal cells. Non-neuronal cells had very low activity of choline acetyltransferase (CHAT); addition of serum along with NGF resulted in a marked increase in CHAT. A significant increase in CHAT was observed when SN were co-cultured with heart or liver cells, but not skeletal muscle cells. The activity was not elevated along with SN grown in a medium conditioned by the heart cells. These results suggest that various types of cells secrete trophic factor(s) that specifically enhance the noradrenergic character of the NGF-dependent SN of the chick embryo.
PERIPHERAL NERVE IMPLANT EFFECTS ON SURVIVAL OF RETINAL GANGLION CELL. S. Schmitz* and J.R. Della, William P. Arnold Pain Treatment and Research Center, New England Deaconess Hospital, and the Charles A. Dana Research Laboratories, Departments of Neurology, Beth Israel Hospital and Harvard Medical School, Boston, MA 02115.

We have previously reported (Delfs and Saroff, Soc.Neurosci.Abstr. 36:145, 1986) the culturing of rat spinal cord muscle fibers along with pretreatment of muscle fiber technique of Gahwiller (Neuroscience 11: 751, 1984). Using this technique, we have established cultures of neonatal rat skeletal muscle fibers alone and cocultures of rat skeletal muscle fibers with transverse slices of neonatal spinal cord.

When teased skeletal muscle fibers were cultured alone, many of the explanted muscle fibers remained histologically unchanged, with no outgrowth or myotube formation being seen only rarely (<1%). In those rare cultures of muscle alone in which some regeneration was observed, development progressed only to the myotube stage, and in no case was development into mature muscle fibers observed.

On the other hand, when explanted muscle fibers were cocultured with transverse slices of spinal cord, regeneration of explanted skeletal muscle fibers occurred much more often (>40%). In many of the cocultures, but in none of the cultures of muscle alone, the formation of nearly mature muscle fibers with spontaneous contractions was observed within 1-3 weeks. Thus, we have demonstrated (1) that skeletal muscle can be grown using the roller tube technique of Gahwiller, that cocultures of skeletal muscle fibers with spinal cord can be established and maintained, and (3) that in vitro skeletal muscle regeneration is enhanced by the presence of cocultured spinal cord in these organotypic cultures.

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NGF and T³ increase activity of cholinergic enzymes in cultured septal slices B.H. Gehringer* and A. Enz Brain Research Institute, University of Zurich, Zurich; Psychomotor Research, Sandzé Ltd., Basel, Switzerland and Department of Neurology, University of Miami, FL 33130.

Spletal slices derived from 5-day-old rats were cultured either alone or together with hippocampal slices. Choline acetyltransferase (ChAT) determined after 3–4 weeks in vitro averaged 6.1 fold T³ monounsaturated (T³) resulted in concentration-dependent increases in the activity of ChAT. NGF consistently enhanced ChAT activity up to 20-fold, whereas T³-induced effects reached a maximum (5-fold increase) at a concentration of 50 ng/ml and declined with higher concentrations. Equivalent effects were observed in both in vitro preparations. Thymoerin releasing hormone (up to 1 μg/ml) was without any effect on the activity of ChAT. The actions of NGF and T³ were additive. In concentrations which had no influence on cholinergic enzymes per se, T³ still enhanced the stimulatory effect of NGF. All actions on ChAT-activity were paralleled by similar increases in acetylcholinesterase activity. Antibodies to NGF failed to alter basic enzyme activity and T³-induced effects, but completely abolished the action of NGF in mono- as well as co-cultures.

The biochemical observations were consistent with histochemical findings. In septal cultures, application of NGF and T³ strongly enhanced the intensity of staining for AChE and ChAT without inducing radial outgrowth of fibers from the slices. In co-cultures, both substances increased the number of cholinergic fibers that invaded the hippocampal tissue.

Morphological and biochemical effects of nerve growth factor and monosialoganglioside GM1 on septal cells in culture. R.L. Kengisberg, B. Werning and C. Cuello. Department of Pharmacology and Therapeutics, McGill University, 865 Drummond St. Montreal H3G 1V6 Quebec, Canada.

The effects of nerve growth factor (NGF) and monosialoganglioside GM1 alone and in combination were examined in primary dissociated septal cells in culture. Morphological and biochemical parameters were assayed after various periods in co-culture with these agents.

In primary dissociated septal cells were prepared from rat fetal (X 17) septum. The cells were seeded at low (1.5 x 10⁵ cells/ml) and high (3 x 10⁶ cells/ml) densities on poly-L-lysine coated culture dishes. The culture media did not contain any cytotoxic factor inhibitors therefore providing us with a mixed neuronal-glia cell population.

The percentage of surviving neurons was determined immunohistochemically with the use of a monoclonal antibody raised against neurofilaments (Lawson, S.M. (et al.), J. Comp. Neurol. 228, 263, 1984). In addition, the cholinergic neurons were identified with the use of monoclonal antibodies raised against choline acetyltransferase (ChAT) (Eckenstein, P. and Thoenen, H., Nature, 263, 363, 1983). The effects of NGF and GM1 on fiber outgrowth, neuronal survival and nerve growth factor receptor distribution was investigated immunohistochemically (Chandler, C.E. et al. J. Biol. Chem. 259:6882, 1984) both at the light and electron microscopic level. Quantitative parameters (cell number, cell shape and size, number and length of neurites) were derived with the use of image analysis (Quantimet 920).

The exposure of the septal cells to the NGF (100 ng/ml) resulted in a 2 to 3 fold increase in ChAT enzymatic activity. GM1 at a concentration of 30 μg/ml was found to increase the specific activity of ChAT less dramatically than NGF, but was found to enhance fiber outgrowth when compared to control cultures. Concentration of GM1 above 50 μg/ml proved to be cytotoxic. There were no obvious to be differential effects of these two factors which depended upon the cell seeding density of the septal cells in culture.

In conclusion, although NGF and GM1 were effective in increasing ChAT activity and neurite density it appears that they may act by different mechanisms.

This research was supported by grants from NII, Fidia and Medicipor.
TARGET REGULATION OF NEUROTRANSMITTER PROPERTIES IN RAT SYMPATHETIC NEURONS: EVIDENCE FROM SWEAT GLAND TRANSPLANTATION.

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Most sympathetic neurons are adrenergic, however a minority are cholinergic. The best characterized cholinergic sympathetic neurons are those which innervate eccrine sweat glands in the footpad of the rat. Although the adult innervation is cholinergic, the innervation of the foot eccrine sweat gland is initially displays catecholaminergic properties, such as catecholamine fluorescence and tyrosine hydroxylase (TH) immunoreactivity (IR). As development proceeds, these adrenergic traits are gradually lost, while the innervation begins to acquire choline acetyltransferase (ChAT) activity, acetylcholinesterase (AChE) activity and vasoactive intestinal polypeptide (VIP) immunoreactivity, properties of the adult cholinergic innervation.

To investigate the possible role of the target, the sweat gland, in this developmental plasticity, transplantation experiments have been performed in neonatal Lewis inbred rats. Hairy skin, in the lateral thoracic region, was replaced with sweat gland-containing footpad skin. Hairy skin normally receives adrenergic sympathetic innervation, especially the plexicortex, but not cholinergic sympathetic innervation. The sweat gland-containing footpad skin normally receives predominantly cholinergic sympathetic innervation. This paradigm thus allows sympathetic neurons, which would normally innervate adrenergic targets in the hairy skin, to innervate sweat glands instead.

The innervation of the transplanted sweat glands was examined from 8 to 40 weeks post-transplantation (PT). Catecholamine fluorescent fibers first invaded the transplanted glands at 2 weeks PT, and formed an intensely fluorescent plexus around the glands by 3 weeks PT. In contrast, the only faintly fluorescent fibers were observed. The appearance and subsequent decrease in TH-IR followed the same course. AChE was first seen at 3 weeks PT, became more intense by 4 weeks PT, and was maintained at 5 and 6 weeks PT. VIP-IR appeared in the innervation of many glands at 4 weeks PT, and became more intense at 5 and 6 weeks PT. Only occasional Substance P and Neuron Peptide Y fibers were evident at all timepoints examined. ChAT activity was assayed by synthesis of acetylated footpads. Low levels of ChAT activity, 7.4 pmol/mg prot/min, were present at 3 weeks PT, the first timepoint examined, and had increased to 33.2 pmol/mg prot/min at 6 weeks PT.

These results suggest that the transplanted sweat glands are capable of regulating neurotransmitter phenotype in sympathetic neurons, in that target appropriate properties are observed in neurons that would not normally express these traits.

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LOCALIZATION OF NEUROCHEMICAL PROTEINS BY IN SITU HYBRIDIZATION. C. Bandtlow*, R. Heumann*, H.-E. Schaab* and B. Thoenen, Dept. of Neurochemistry, Max-Planck-Institute for Psychiatry, Munich, FRG, and Dept. Neurobiological Institute, Stanford University, Stanford, USA.

In situ hybridization experiments have been able to identify the cell types synthesizing mRNAGF. NGF is an extremely rare message, present at least at one copy per million molecules of polyA-MNA. It was therefore necessary to use probes with high, specific activities, either 32P or 35S-labeled. We chose 32P-labeled probes because they give better resolution than is possible with 35S. The disadvantage of 32P is that it often results in high non-specific background, thereby precluding detection of rare messages such as MNA. Thus, an improvement of available techniques was required. Prehybridization with unlabelled thioαUTP at pH 5.5 normally necessitated the use of probes with high specific activities, whereas mRNAGF levels fell only after the first postnatal week, suggesting that the total NGF supply is negligible. However, after lesioning of the sciatic nerve there was a rapid increase in the mRNA of NGF in the sciatic nerve of rats. We have adopted the working hypothesis that the observed influence of sym patheticectomy is mediated locally through the target tissue since a direct synaptic connection between the CG and the SCG does not exist. We have investigated the role of the target, the sciatic nerve, in the development of the NGF response to sympathetic stimulation.

In the in vivo situation, where after nerve lesion macrophages accumulate near the lesion, a fraction of the NGF message is expressed only during earlier developmental stages, suggesting that mRNA is down regulated with the regression into earlier developmental stages. We have therefore investigated the developmental changes in the levels of mRNA and mRNA in rats postnatal week, whereas mRNA levels fell only after the first postnatal week, reaching adult levels by the third week. At birth the message of mRNA and mRNA in the sciatic nerve were 10-20% the levels of the adult mRNA, whereas mRNA levels were those of the adult mRNA. Following a crush lesion (allowing regeneration), changes in the mRNA levels were similar to those in vivo, whereas for mRNA only the initial rapid increase was observed. This in vivo situation only when the nerve is activated macrophages were found in the sciatic nerve, whereas in vivo in situ hybridization indicated that mRNA and mRNA in in situ hybridization were found in the sciatic nerve, whereas in vivo in situ hybridization indicated that mRNA and mRNA were restricted to the sym pathetic nerve, whereas mRNA was present in both the sympathetic and parasympathetic regions of the sciatic nerve.

(Supported by the Medical Research Council of Canada)


In situ hybridization with unlabelled thioαUTP at pH 5.5 and the substitution of DTT with the more heat stable B-mercaptoethanol were required. The advantage of 32P-labeled probes was that it allowed the identification of rare messages such as MNA. Thus, it was necessary to use probes with high, specific activities, either 32P or 35S-labeled. We chose 32P-labeled probes because they give better resolution than is possible with 35S. The disadvantage of 32P is that it often results in high non-specific background, thereby precluding detection of rare messages such as MNA. Thus, an improvement of available techniques was required. Prehybridization with unlabelled thioαUTP at pH 5.5 normally necessitated the use of probes with high specific activities, whereas mRNAGF levels fell only after the first postnatal week, suggesting that the total NGF supply is negligible. However, after lesioning of the sciatic nerve there was a rapid increase in the mRNA of NGF in the sciatic nerve.

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446.4 DEVELOPMENT OF THE CHICK TRIGEMINAL REGION: 125I-NGF BINDING, HNK-1 AND NEUROFLAMENT IMMUNOREACTIVITY.
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This study was a continuation of our investigations of the developing trigeminal (V) system in the chick embryo. Previous work in our lab has shown that in vitro, trigeminal motoneurons fail to extend their axons in the absence of the trigeminal ganglion. In in vivo studies on the effects of ganglion conditioned medium on neural tube explants containing the V motoneuron population led to the observation that NGF was able to increase neurite outgrowth from these preparations.

Using 125I-NGF we have examined NGF binding in the trigeminal region. We have been binding this region as early as embryonic day 4. There is binding in the ganglion and along the peripheral nerve. Heaviest binding is at the point where the sensory fibers enter and the motor fibers exit the brainstem, the equivalent of the dorsal root entry zone. There also appears to be labeling of the early motoneuron cell bodies once they accumulate in the lateral brainstem. The motor root courses through the medial aspect of the trigeminal ganglion does not appear to heavily bind NGF as the sensory fibers do where they accumulate to enter the brainstem.

HNK-1 and neurofilament antibodies (provided by M.Bronner-Fraser and G.Beaumont respectively) were used to more fully describe the disposition of fibers and cell bodies in the trigeminal region. Both HNK-1 and the neurofilament antibodies confirm that the entry zone which was so heavily labeled in the NGF binding study consists exclusively of accumulated fibers. The entry zone region of the brainstem can be identified at 2 days of incubation by the accumulation of migrating neural crest cells at this point. This is clearly seen with the HNK-1 antibody.

Currently, the observations on NGF binding in this region are being extended to include earlier time points. (Supported by NIH grant NS-20387 to M.B.H.)

We previously reported that destruction of cholinergic afferents from basal forebrain nuclei induces abnormal cortical morphogenesis in the Balb/c mouse. Unilateral electrolytic lesions were placed, at birth, in the magnocellular basal forebrain nucleus [nBM] which project to frontal and parietal cortex. A severe disruption of cortical cytoarchitecture resulted, ipsilaterally to the lesion, in all areas displaying reductions of acetylcholinesterase [ACHE] histochemistry. Lesions of nonneurotropic, somatostatinergic or thalamocortical afferents did not produce comparable developmental abnormalities.

The lesion-induced abnormal cytoarchitectonic pattern most likely resulted from a delay in neuronal differentiation. Cells in several cortical layers retained an undifferentiated appearance, ipsilateral to the lesion, much longer than in the contralateral hemisphere, but contralaterally neurons formed a more differentiated appearance concurrent with the recovery of cholinergic markers. In the adult the most prominent abnormality was an absence of a distinctive cortical layering ipsilateral to the lesion, further indicating that neuronal death occurs in the lesion-affected hemisphere. The delayed development of a lesion-induced delay in neuronal maturation is correct or whether other events, such as abnormal cortical cell migration or neuronal loss in cortex, may occur. To this end we have monitored the fate of neurons labelled by [3H]thymidine (3H-Thy) at the time of their generation. Further, we assessed the neurochemical development of GABAergic and somatostatin [SOM] neurons intrinsic to frontoparietal cortex.

Time-mated dams were injected i.p. with three daily doses of 5uCi of [3H-Thy] on gestational days E16,17, and 18. Their pups received unilateral nBM lesions at birth, were formalin perfused on PDS or PD30, sectioned frozen at 10um and processed for autoradiography. Glutamic acid decarboxylase [GAD] enzyme activity was measured to assess GABA synthesis in cortical neurons. Further, SOM neurons were monitored in cortex immunohistochemically. Neurons not expressing SOM by [3H-Thy] incorporation on E16,17 or 18 were found in the same layer (layers III and IV) in both lesioned and control cortex. The amounts of labelled cells in the two hemispheres were also comparable. Thus, this cell migration appears to proceed normally despite the lesion and cell numbers at least in layers III and IV of cortex were not affected. No difference in GAD activity and, intrinsic to cortex, SOM-IR cells could be found between (ipsilateral to lesion) and control, further indicating that neuronal death is an unlikely cause for the cortical maldevelopment. However, a transient population of SOM-IR neurons were monitored in cortex immunohistochemically. Neurons labelled to some small extent in layers HI and to some small extent IV) in ipsilateral to lesion and contralateral cortex.

The present results are compatible with the hypothesis of maturational delay as the consequence of cholinergic denervation at birth. In addition, the absence of differences in cortical GAD activity suggests that the neurochemical development of GABAergic and somatostatin intrinsic to frontoparietal cortex. The amounts of labelled cells in the two hemispheres were also comparable. Thus, we have monitored the fate of neurons labelled by [3H]thymidine (3H-Thy) at the time of their generation. Further, we assessed the neurochemical development of GABAergic and somatostatin [SOM] neurons intrinsic to frontoparietal cortex.

As reported in the preceding abstract, distribution and numbers of somatostatin immunoreactive cells were not markedly changed within corticocortical displays AChE depletion and cytoarchitectonic abnormalities. However, we observed a transient increase in the number of SOM-IR neurons in the alveolar and orietes layer of the hippocampus, ipsilateral to the lesion, between PN 7 and 11. During the same time period, the transient population of SOM cells appeared ipsilaterally, in the sub-cortical white matter, 1-3 weeks following the lesion. We conclude that neuronal death is an unlikely cause for the cortical maldevelopment. We have monitored the fate of neurons labelled by [3H]thymidine (3H-Thy) at the time of their generation. Further, we assessed the neurochemical development of GABAergic and somatostatin [SOM] neurons intrinsic to frontoparietal cortex.

The present study shows a close temporal and spatial correspondence in the appearance of SOM-IR neurons and the gene expression of SOM in the telencephalon of the mouse. Furthermore, our results suggest that the impact of nBM lesions on the development of neuronal populations is even more extensive than our previous results indicated. Supported by: PO1 HD19920, ROI HD19920 and the McKnight Foundation.

Classically, γ-aminobutyric acid (GABA) acts as a major inhibitory neurotransmitter in the mature central nervous system (CNS). Recent evidence indicates that GABA plays a role in the development of its synaptic contacts. We have studied the relationships between the various biochemical components of the GABA system, using the rabbit retina as our model. If the activity of the uptake system, which removes endogenous GABA from the synaptic cleft, is blocked during development by either an in vivo or in vitro treatment with nipeptic acid, a GABA analogue; an increase in the number of GABA receptors results. This “up regulation,” or “induction,” of receptors only occurs in immature tissue since treatment of mature tissue results in a loss of receptors. This sensitivity to nipeptic acid is maximal around the time of eye opening and is light-dependent. While this induction has been shown to involve the GABA receptor, since treatment with GABA agonists mimics this effect; the mechanism by which it acts is unclear. This question has been addressed in this study. Newborn rabbit pups were delivered to the laboratory one day after birth and sacrificed. Eyecups then were prepared and incubated in the presence of various GABA analogues in order to determine the specificity of action. Co-incubation of nipeptic acid and THIP (a GABA agonist) resulted in an increase in binding equal to that obtained after treatment with either alone. This non-additive effect suggests that both act through the GABA receptor. Similar treatments did not increase low-affinity binding. In addition, the time required for the effect to appear was studied by incubating the tissue with nipeptic acid for various time periods. The level of binding was measured by 3H-muscarnic ligand binding to prepared membranes. An increase in high-affinity binding was observed as early as 25 minutes after treatment with nipeptic acid began. This suggests that the receptors increase as a result of exposure of pre-existing receptors rather than de novo synthesis. Thus, blocking uptake by nipeptic acid probably raises synaptic levels of GABA, increasing the time of interaction of GABA with its high-affinity receptors. This would result in a rapid exposure of high-affinity receptors on the surface of immature membranes. These results further support the suggestion that GABA acts as a trophic factor in the developing CNS. Funded by a grant from Research Corp.
450.1 ESTRADIOL INCREASES SPONTANEOUS ACTIVITY OF HIPPOCAMAL NEURONS IN CULTURE. J.R. Meyer and R.Robbins. Deps. of Internal Medicine, and Obstetrics and Gynaecology, Yale University School of Medicine, New Haven, CT, 06510.

Steroid influences upon neuronal function have classically been thought to result from genotypic effects, which appear at low latencies and last for extended periods following administration of a steroid. There is increasing evidence, however, that steroids also exert non-genotypic effects upon neuronal activity. Such effects may exist in the hippocampus, where estradiol 17β (E2) causes short-latency immediate early responses (Huyer et al. Science 209: 1017). In a preliminary effort to understand the mechanism of E2's effect upon the hippocampus, we assessed the influence of the activity of hippocampal neurons in dissociated cell cultures from newborn rats.

Recordings were made from 2 to 5 week old cultures in a static bath containing 2 ml of a phosphate buffered saline solution with 14 gelled (estrogen-free) horse serum. A modified cell-attached patch clamp technique was used to record spontaneous action potentials from individual cells. Mean discharge frequencies were obtained over a 5 minute initial period, after which 18 ml of a test solution was bath applied. 3 test solutions were used: phosphate buffered saline (PBS; control group), and a solution in PBS containing E2 at 10-6 M, with or without E2 and forskolin groups, respectively: PBS test solution concentration=0.24, final saline bath concentration=0.0014; E2 test solution concentration=7.2 x 10^-4 M, final saline bath concentration=1.6 x 10^-4 M. Discharge frequencies over the subsequent 10 minute experimental period were obtained and the mean frequency over this period normalised to the mean frequency of the initial period (normalised frequency).

Both steadily-discharging and bursting cells were encountered. For all cells (n=70), the mean frequency over the initial period was 0.93±0.05 Hz (s.e.m.). In the control group, firing frequencies during the experimental period showed no apparent changes from the initial period of normalised frequency (E2p=0.13; r=0.28). A similar finding was noted among the ethanol group (E2=0.10±.02; E2p=0.10; r=0.12). In the E2 group, however, spontaneous activity displayed increased (N=6) or decreased (N=6) significantly different from the changes seen in the control and ethanol groups (p<0.05; T-tests). The change in frequency was noted 3 minutes following E2 application, with lesser increases over the remainder of the experimental period.

The specificity of estradiol 17β's effect upon hippocampal cells is to be determined, as does the mechanism of estradiol's action. Such studies will be important for understanding of increased seizure activity related to high estrogen states. Supported by NIH grants HD35978 and NS06206.


Recent studies have demonstrated that: (1) estradiol (E2) alters the pattern of release of noradrenalin and norepinephrine in the hypothalamus of ovx (OVX) rats; (2) NE can increase the density of its own receptors; and (3) in the brain, β1 and β2 adrenergic receptors exhibit a rhythm in frontal cortex. Therefore, we wished to determine whether β1 and β2 receptor densities would exhibit a diurnal rhythm in OVX rats and whether E2 treatment alters this rhythm in discrete brain regions that control reproductive function.

Using quantitative autoradiographic methods, we measured a decrease in β2 receptors in discrete brain regions that control female reproduction (hypothalamus, medial preoptic area, and median eminence) in female rats treated with E2. The data demonstrated that E2 may alter the control of reproductive function by altering receptor densities. Supported in part by NIH grant HD2262.
MELATONIN INCREASES SERUM INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) IN MALE SYRIAN HAMSTERS. J. Friend*, M. S. Sheppard* and S. M. Hala*. (SPON: B. J. Devine). Dept. Pharmacology, Univ. of Toronto, Toronto, Ont. M5S 1A8, Canada.

Daily melatonin injections produce a syndrome in male Syrian hamster which includes gonadal atrophy, reduced levels of circulating thyroid hormones, and weight loss in body weight. The objective of this study was to determine the effects of daily melatonin injections on circulating levels of the growth factor, IGF-1, in male Syrian hamster. Melatonin (50 μg/kg) was administered daily subcutaneously (25 micrograms in 0.1 ml saline) every day for six weeks to normal male hamsters (n=8) maintained on a 14L:10D photoperiod. Melatonin was administered to hamsters receiving 5 micrograms thyroxine subcutaneously (in 0.1 ml saline) every other day. All injections were administered 1-2 hrs before lights out. The data were analyzed with the mixed model of the linear model function of Systat 4.0. The results showed that melatonin treatment had significant effects on body weight and serum IGF-1 concentrations (p < .01). Melatonin treatments significantly elevated IGF-1 levels (p < .001).

These data suggest that melatonin influences body weight via an IGF-1-dependent mechanism that differs from the mechanisms by which thyroid hormones influence body weight. The melatonin-induced increase in serum IGF-1 could be associated with a loss in gonadal and thyroid hormone secretion. This explanation is consistent with a CNS site of action of melatonin. An alternative explanation is that melatonin acts directly on IGF-1 secreting cells. (Supported by the Medical Research Council of Canada.)

CORTICOTROPIN-RELEASING FACTOR IMMUNOREACTIVITY IS REDUCED BY EITHER GONADECTOMY OR CHRONIC ESTROGEN TREATMENT. D.A. Haas, B. Borgundvaag and J.R. George, Departments of Medicine and Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8, Canada.

Corticotropin-releasing factor (CRF) has been implicated in hypothalamic modulation of the reproductive axis. However, since the role of gonadal steroids in the regulation of CRF is not known, this study was undertaken to test this hypothesis. Hypothalamic CRF-like immunoactivity (CRF-ir) was determined using a radioimmunoassay specific for rat CRF. Adult male rats underwent either orchidectomy or sham surgery. Two weeks later the orchidectomized rats were administered testosterone propionate (20 mg/kg/day), or vehicle for 1 or 3 days while the sham-treated group was given vehicle (n=7 per group). Orchidectomy resulted in a significant (p<0.025) decrease in CRF-ir compared to sham-treated rats (100±16.6 pg/mg vs 138.9±4.6 pg/mg). Testosterone administration did not alter CRF-ir levels significantly from vehicle treatment in orchidectomized rats. Adult female rats sacrificed 3 weeks after orchidectomy (n=15) were found to have significantly (p<0.025) decreased CRF-ir compared to intact females (n=15) (84.5±8.3 pg/mg vs 118.7±8.5 pg/mg). Administration of β-estradiol-3-Gonadotropin (E) (50 μg/day, s.c.) for periods up to 5 days did not significantly alter CRF-ir in orchidectomized rats. In order to study the effects of chronic estrogen administration, female rats were orchidectomized and surgically implanted with silastic pouch containing 2.5 mg E (n=4) or no hormone (n=4) at 3, 8 or 12 weeks after surgery. CRF-ir was significantly reduced in the E-treated groups at 3 (100±32.5 pg/mg vs 67.2±5.3 pg/mg, p<0.005) and 12 weeks (90.4±12.5 pg/mg vs 46.3±14.3 pg/mg, p<0.01), with no significant difference noted at 8 weeks. Intact females which were vehicle treated for 12 weeks, did not differ from females receiving saline (51.8±13.0 pg/mg). These data show that hypothalamic CRF-ir was reduced by gonadal hormones in both male and female rats. Chronic treatment with gonadal steroids could not reverse this decrease in either sex. Chronic treatment of E decreased CRF-ir even further in orchidectomized rats, but E alone decreased CRF-ir in intact females. The combination of these results imply that the gonadectomy-related increase in CRF-ir was not directly mediated by the loss of gonad steroids, but also by some other factor, possibly gonadal in origin.

CORTICOTROPIN-RELEASING HORMONE AND OXYTOCIN STIMULATE THE SECRETION OF IMMUNOACTIVE P-ENDORPHIN FROM HUMAN PLACENTA IN VITRO. A. M. Margolis*, W. Grinb*, P. Protos*, F.W. Gold and G.P. Chrousos. (SPON: J.R. Glowa) DEB, NICHD, NIH and BPB, NIN, Bethesda, MD 20892, and Holy Cross Hospital, Silver Spring, MD 20901

Plasma immunoreactive (IR) corticotrope releasing hormone (CRH) concentrations increase progressively during pregnancy and rise further during labor. At parturition plasma immunoreactive oxytocin (OT) also rises significantly. Immunoreactive P-endorphin and IR-CRH are present in human placental extracts. P-endorphin-like mRNA has been described in extracts of human placenta and we have recently isolated a cDNA clone for corticosterone receptor, whereas CRF receptor may be modulated by association of B with the type 111, glucocorticoid receptor. (Supported, in part, by AMR112, HHS11, and a UCSF Academic Senate Research Grant. SPA also expresses appreciation to the Kroc Foundation.)

To investigate the relationship of CRH, OT, and placental IR-beta-endorphin we have developed an in vitro perfusion system utilizing fresh human term placentas fragments. After a 60 min equilibration period the rate of peptide secretion was constant for at least 4 hours. The secretion ratio of IR-CRH was 135±25.5 (mean ± SD, n=6) pg/5 min/fraction/g tissue and of IR-beta-endorphin 7.2±1.3 (n=6) pg/5 min/fraction/g tissue. The ratio of secretion of IR-CRH to IR-beta-endorphin is dramatically greater than in term placental extracts. In order to study the effects of chronic estrogen administration, female rats were orchidectomized and surgically implanted with silastic pouch containing 2.5 mg E (n=4) or no hormone (n=4) at 3, 8 or 12 weeks after surgery. CRF-ir was significantly reduced in the E-treated groups at 3 (100±32.5 pg/mg vs 67.2±5.3 pg/mg, p<0.005) and 12 weeks (90.4±12.5 pg/mg vs 46.3±14.3 pg/mg, p<0.01), with no significant difference noted at 8 weeks. Intact females which were vehicle treated for 12 weeks, did not differ from females receiving saline (51.8±13.0 pg/mg). These data show that hypothalamic CRF-ir was reduced by gonadal hormones in both male and female rats. Chronic treatment with gonadal steroids could not reverse this decrease in either sex. Chronic treatment of E decreased CRF-ir even further in orchidectomized rats, but E alone decreased CRF-ir in intact females. The combination of these results imply that the gonadectomy-related increase in CRF-ir was not directly mediated by the loss of gonadal steroids, but also by some other factor, possibly gonadal in origin.
PLATELET ACTIVATING FACTOR STIMULATES HYPOTHALAMIC CORTICOTROPIN RELEASING HORMONE SECRETION IN VIVO


Products of the immune system may interact with the hypothalamic-pituitary-adrenal (HPA) axis. During allergic or inflammatory reactions, platelet-activating factor (PAF), a glycoprotein with potent platelet-aggregating properties, is released by neutrophils, eosinophils and basophils. To examine the hypothesis that PAF contributes a link between the immune system and the HPA axis, we investigated the ability of this chemical to stimulate immunoreactive corticotropin releasing hormone (CRH) secretion by explanted rat hypothalamus cultured in vitro. Simple explants of rat hypothalamus were incubated overnight in medium containing 0.1% BSA and 20% fetal calf extract. The following day the hypothalami were exposed to PAF at concentrations ranging from 10^{-6} M to 10^{-2} M for 40 min. Each hypothalamus was finally exposed to 60 kU ECL to test the viability of the tissue. A minimum of 6 hypothalami per group were examined. ANOVA and Dunnett multiple range test were performed on logarithmically transformed data. PAF stimulated CRH secretion at concentrations ranging between 10^{-6} M to 10^{-2} M. The most effective concentration was 10^{-5} M (p<0.01 vs basal secretion), inducing a 200% increase in CRH above baseline values. To examine whether prostanoids are involved in PAF-induced CRH response, we evaluated the ability of indomethacin, a cyclooxygenase inhibitor, and eicosatetraycic acid (ETYA), a cyclooxygenase inhibitor, to inhibit this response. Both of the inhibitors were added 2 hr prior to and during treatment with 10^{-5} M PAF. In addition, aspirin and diazepam, two known inhibitors of CRH secretion, were added separately to media 20 min before treatment and during exposure to 10^{-5} M PAF. The PAF-induced CRH secretion was completely prevented by treatment with indomethacin and ETYA (p<0.01 vs 10^{-5} M PAF). In contrast, aspirin (10^{-4} M), but not by NGCA, aspirin (10^{-4} M), but not diazepam, also inhibited PAF-induced CRH secretion (p<0.01). In summary, PAF by itself elicited a single, explanted rat hypothalamus. This action may represent another link between the immune and the endocrine system. The PAF-induced CRH secretion is probably mediated by prostaglandins, but not leukotrienes, and can be blocked by aspirin or diazepam.

To further explore the relationships between CRH and PAF in the hypothalamus, we investigated their effects on CRH mRNA levels. Previous studies have demonstrated that CRH mRNA is selectively expressed in the paraventricular and supraoptic nuclei (PVN and SON) of the hypothalamus. To examine the effect of these neuropeptides on CRH mRNA levels, we used in vitro hybridization histochemistry. These data suggest that the increased CRH mRNA expression is mediated by PAF, acting through a mechanism that involves the inhibition of cAMP-dependent protein kinase (CaMPK) activity. These results indicate that PAF may play an important role in the regulation of CRH secretion, potentially through its effects on the hypothalamic CRH secretory neurons.
TEMPORAL INTERACTIONS INVOLVED IN DIRECTION-SELECTIVITY OF STRIATE CORtical NEURONS OF THE CAT. Curtis L. Baker, Jr. and Max E. Cyander, Department of Psychology, New York University, 205 Ave. Docteur Penfield, Montreal, Quebec, Canada, H3A 1B1; and Dept. of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4R2

Flashed bar-shaped stimuli were used to study direction-selective neurons in striate cortex of the cat. Stimuli flashed sequentially at two adjacent points in the receptive field produced direction-selectivity comparable to that elicited by continuously moving stimuli.

Previous studies have shown that each neuron has a characteristic optimum spatial displacement (Dopt) between the 2 flashes to produce maximal direction-selectivity. This value of Dopt was shown to be independent of the temporal interval used in its measurement, and linearly proportional to the spatial subunit wavelength (reciprocal of best spatial frequency) for a given neuron. Attempting to optimise temporal separation between 2 flashes was frustrated by the finding that most striate cortical neurons remained only to prolonged flash exposure durations. A given neuron's "integration time" was found to be inversely proportional to Dopt and to the spatial subunit wavelength. The optimum velocity for direction-selectivity in a given neuron appears to be jointly determined by the spatial property (Dopt or spatial frequency) and the integration time.

To probe the temporal conditions needed for 2-flash direction-selectivity, a neuron's directional response was measured for a series of values of temporal stimulus onset asynchrony (SOA) between 2 flashes delivered at a constant spatial displacement (Dopt measured for that neuron). These measurements were made for a series of values of flash exposure durations, with all experimental conditions randomly interleaved. Some neurons exhibited direction-selectivity for a broad range of SOAs with little dependence on flash exposure duration. Other neurons exhibited some selectivity for a constant interval between the end of the first flash and the beginning of the second flash. Correlation of this qualitative heterogeneity with other cell properties is not yet evident.

These data may be interpreted in terms of different kinds of input signals (e.g., transient vs sustained) to Reichardt-type correlation models and suggest that direction-selectivity in striate cortex neurons may arise independently from nonlinear directional interactions between several different combinations of inputs.


In striate cortical receptive fields of the cat, we have analyzed nonlinear response interactions between pairs of optimally oriented bars that represent samples of movement. Pairs were drawn from a maximised spatial and temporal bar sequence that included a wide range of separations in space (x) and time (t) to test a wide range of possible velocities. Direction-selective striate neurons display nonlinear facilitation for responses to movement sequences in the preferred direction and nonlinear suppression for responses in the null direction.

To gain insight into the connectivity of neurons performing this test, we simulated a two-bar superposition test for two different models that have been proposed to explain movement processing, the Reichardt model and the opponent energy model. Our measured nonlinear interaction disagreed with the nonlinear residual of the simulated superposition test at the final "opponent" output of both models. The measured cortical interaction for one movement direction did not always invert for the sequence with x and t values of the same magnitude (same equivalent speed), but in the opposite direction, as required by the models. This same disagreement existed between measured cortical interactions and simulated interactions at the half-detector stage of the Reichardt model, the stage immediately preceding the opponent output, because spatial-temporally tuned Reichardt detectors produce an inherent opponency even before the final output stage.

However, the lack of opponency in the measured interactions agreed closely with the simulated interaction for the preponent stage of the energy model, the stage at which separate "energy" for opposite directions are combined. The Reichardt model simulation agrees with our finding that in directionally selective striate neurons the x for maximal interaction is proportional to the area of motion. The interaction is highly inseparable in space and time, and shows a strong velocity preference.

Thus, our directionally selective cortical neurons match closely the properties at the preponent stage of the energy model. This observation (1) that cortical responses to bidirectional motions do not carry fully motion-opponent information, and (2) that these cells may depend on strongly inseparable peripheral filters that are similar to spatial-temporal Gabor filters. (Supported by RO1630, EV06579, and EY01319.)

ADAPTATION OF SINGLE UNITS IN CAT VISUAL CORTEX: AFTEREFFECTS AS A FUNCTION OF DIRECTION, WITH IMPLICATIONS FOR CORTICAL ORGANIZATION. Saul A.B. and Cyander, M.S. Department of Psychology, Dalhousie University, Halifax, NS, Canada, B3H 4R2

We employed an adaptation paradigm in which each of 5 second stimulus presentations was immediately preceded by 15 seconds of adapting stimulation. All stimuli were drifting gratings of varying contrast and orientation. The contrasting and spatial properties were shown to be sensitive to aftereffects; in about 100 runs on 25 cortical cells, we observed selective adaptation in every run. The adaptation results have implications for cortical connectivity. The mechanism underlying direction selectivity is believed to involve inhibitory connections. One might suppose that this inhibitory mechanism links between an array of selectivity cells preferring opposite directions of motion. Adapting with a high-contrast grating drifting rightward drastically reduces the responses of cells which prefer rightward motion; if these cells normally inhibit a cell preferring leftward motion, rightward adaptation would reduce this inhibitory input. The disinhibition would show up as an enhanced test response in a cell's null direction following adapting in the null direction. We have found, to the contrary, that adaptation aftereffects are bidirectional, and have never observed enhanced responses in the null direction following adapting in the null direction.

These bidirectional aftereffects are also temporal frequency selective. The tuning of adaptation in the temporal domain is often surprisingly sharp, with octave boundaries. Both directions of motion have similar tuning of adaptation, although sometimes the magnitude of the aftereffect is reduced in the direction opposite to that adapted. We have found that the tuning of aftereffects depends on the adapting stimulus, implying that the mechanisms underlying adaptation are specific to a cell, rather than intrinsic properties of the cell alone. Since aftereffects are bidirectional and temporal frequency selective, we suggest that direction-selective cells receive bidirectional, temporally tuned inputs. Direction selectivity can be generated from such inputs through spatiotemporal specific interactions. In experiments where we varied the adapting contrast, the contrast threshold for reducing aftereffects matched the cell's contrast threshold. The results of these experiments can be used to argue for inhibitory mechanisms underlying adaptation. Modification of the gain can give an explanation for why adaptation reduces cortical responsiveness without inducing a compensatory disinhibitory response.


In striate cortical receptive fields of the cat, we have analyzed nonlinear response interactions between pairs of optimally oriented bars that represent samples of movement. Pairs were drawn from a maximised spatial and temporal bar sequence that included a wide range of separations in space (x) and time (t) to test a wide range of possible velocities. Direction-selective striate neurons display nonlinear facilitation for responses to movement sequences in the preferred direction and nonlinear suppression for responses in the null direction.

To gain insight into the connectivity of neurons performing this test, we simulated a two-bar superposition test for two different models that have been proposed to explain movement processing, the Reichardt model and the opponent energy model. Our measured nonlinear interaction disagreed with the nonlinear residual of the simulated superposition test at the final "opponent" output of both models. The measured cortical interaction for one movement direction did not always invert for the sequence with x and t values of the same magnitude (same equivalent speed), but in the opposite direction, as required by the models. This same disagreement existed between measured cortical interactions and simulated interactions at the half-detector stage of the Reichardt model, the stage immediately preceding the opponent output, because spatial-temporally tuned Reichardt detectors produce an inherent opponency even before the final output stage.

However, the lack of opponency in the measured interactions agreed closely with the simulated interaction for the preponent stage of the energy model, the stage at which separate "energy" for opposite directions are combined. The Reichardt model simulation agrees with our finding that in directionally selective striate neurons the x for maximal interaction is proportional to the area of motion. The interaction is highly inseparable in space and time, and shows a strong velocity preference.

Thus, our directionally selective cortical neurons match closely the properties at the preponent stage of the energy model. This observation (1) that cortical responses to bidirectional motions do not carry fully motion-opponent information, and (2) that these cells may depend on strongly inseparable peripheral filters that are similar to spatial-temporal Gabor filters. (Supported by RO1630, EV06579, and EY01319.)


We and others have demonstrated that cells in primary visual cortex linearly summate stimuli distributed in 2D space. However, responses to moving patterns are often highly dependent on the direction of motion. Traditionally, it has been viewed as a spatiotemporal nonlinearity that the energy model successfully accounts for in the null direction. More recent models of motion perception utilize various mechanisms to generate opponency between directions of motion in spatiotemporal space.

In striate cortical receptive fields of the cat, we have developed a variation on such a linear model (after Adelson & Bergen) which accounts for responses of simple cells to moving stimuli. To test this model, we have estimated the 3D (space-space-time) impulse response of simple cells by crosscorrelating the spike train with a white noise stimulus ensemble. This measurement was used to predict the optimal direction and velocity of moving stimuli using the convolution integral. These predictions agree closely with experimentally acquired responses to moving stimuli for the majority of simple cells. A contour plot of a section through the 3D impulse response is shown below. The x-axis is the width of the field which consists of two subregions, one driven by bright stimuli (solid lines) and the other by dark (dashed lines). These subregions, which are elongated, parallel and oriented in the plane of motion, are separated by a bright and dark moving bars. Since orientation of bright and dark subregions was similar, direction preference was the same for both bright and dark moving bars. We conclude that most simple cells in this area are sensitive to the direction of motion in space-time and that it is orientation and speed of the subregions in space-time which confer directionally asymmetric responses. (Supported by NBS-8420402.)
451.5 DIRECTIONAL SELECTIVITY OF SIMPLE CELLS IN CAT STRIATE CORTEX
RESULTS FROM LINEAR SPATIAL SUMMATION. I. S. SPON: A. SCHOPPMANN
In a previous report (Soodak, PNAS 83:1295, 1986) a receptive field model of cortical simple cells was presented which accounted for both the sharp orientation tuning and directional selectivity of these neurons. In this model, simple cell receptive fields are modeled as a linear summation of Gaussian subunits, which can be thought of as representing the responses to stationary contrast-modulated stimuli. The ability of this model to account for the directional selectivity of simple cells suggested that linear spatial summation might be the key to understanding the role of simple cell receptive fields that relate to their directional selectivity properties, and compared these results to predictions of the model.

The experimental paradigm consisted of measuring the directional selectivity of simple cells using sinusoidal gratings and comparing this with the responses to stationary contrast-modulated stimuli. The stimulus was a narrow-band gratings and their contrast was modulated sinusoidally with time. Both response amplitude and response phase were measured as a function of position of the stimulus in the receptive field. Consideration of the dependence of response phase on the position of the stimulus was especially important. In the proposed model, directional selectivity is due to construction of receptive fields from subregions that are not constrained to respond at either 0 deg temporal phase delay (pure on response) or 180 deg temporal phase delay (pure off response). A linear receptive field with this constraint will have invariant position of a stationary contrast-modulated stimulus. In contrast, a receptive field with directional selectivity due to linear spatial summation will show a continuous variation of response phase with position of all the subregions involved in the stimulus. In cells with little or no directional selectivity, the response phase to bar and grating stimulus was relatively invariant with position. Those cells with strong directional selectivity showed continuous variation in response phase with position, and direction of change was always in the predicted direction.

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451.6 DIRECTIONAL SELECTIVITY OF SIMPLE CELLS IN CAT STRIATE CORTEX
RESULTS FROM LINEAR SPATIAL SUMMATION. II. R. CLAY REID, Robert E. SOODAK, and Robert M. SHAPLEY. The Rockefeller University, N.Y., New York.
When stimulated with contrast reversing gratings, any neurally summing directional receptive field (RF) should show continuous variation in response phase as the position of the grating is varied. More quantitatively, it can be shown that the amplitude and phase of the response to drifting gratings would vary with the stimulus phase as a simple functional form, most simply modeled as an ellipse in a polar plot of amplitude and phase. Moving gratings give gratings of the model. Our data from simple cells show an excellent fit to this form, arguing for a high degree of linear spatial summation for non-directional stimuli. Furthermore, we have found by fitting this function, a quantitative prediction of the directional behavior of the model. To test this, gratings of the same spatial frequency, temporal frequency, and contrast as the contrast reversing gratings were drifted in the preferred and non-preferred direction. We have found a very high correlation between the predicted directionality from the contrast-reversing gratings and the response to drifting gratings, often predicting >50% of the actual directionality. Studies parametric in spatial and temporal frequency suggest that the directionality varied with these parameters, often decreasing at higher temporal frequencies, a variation which was mirrored in the prediction from the static gratings.

The linear mechanisms of directional selectivity were robust even in small regions of the RF. First, when the RF was masked except for a narrow window entirely within an "on" or "off" region, the cells still showed directional selectivity to drifting gratings. Even when less than a quarter of a cycle was visible at any time. Second, when static narrow bars (rather than sine-wave gratings) were modulated at low spatial frequency and/or low temporal frequency, the response phase varied continuously across the RF. Studies parametric in spatial and temporal frequency suggest that the response phase change does not seem to be due to simple delays, but to more complex changes in pre- and post-stimulus integration. Therefore, rather than describe the stimulus dependence of on and off regions (even with variable temporal delays), we took a more complex characterization of the temporal characteristics necessary. In conclusion, we have found that the temporal response characteristics of these simple cells vary continuously along the primary visual axis, this variation explains a good deal of the directionality of these cells.

Supported by NIH T32 GM 07739 and EY 1472.

451.7 SENSITIVITY OF CAT CORTICAL NEURONS (AREAS 17, 18, 19, PHMS) TO INTERACTION BETWEEN OBJECT AND BACKGROUND - LOCAL vs. GLOBAL PROCESSING REVISITED. H.B.O. BING and J. SCHLOER, Biophysical Section, Univ. of Mainz, D-6500 Mainz, FRG
While quite a lot is known about how neurons analyze objects in global spatial aspects of visual information processing, far less is known concerning the analysis of object-background interactions between object and background and the contribution of the different visual areas to this task.

Neurons in the visual areas 17, 18, 19 and PHMS were stimulated with black bars defined as an object superimposed with a large field visual noise (2-dimensional Gaussian noise with 50 degrees of freedom for the visual field) defined as background. Both were moved with different relative velocities, inphase or anti-phase to each other.

Using this method, the activity of all neurons tested in each area was affected. It was possible to fit these neuronal effects into a scheme based on the assumption that the following parameters resulting from object-background-interaction have to be determined by neuronal systems: (1) motion, (2) velocity and (3) direction of the background, and (4) relative motion, (5) relative velocity and (6) phase between object and background. Indeed, six types of neurons, each sensitive to one or more of these parameters, were found in all areas tested, though in different percentages.

Systematically taking out different parts of the visual receptive field provided evidence that these parts exert a strong excitatory or inhibitory modulatory effect on the response to the object. Although it is not possible to elicit neuronal activity from these neurons alone, they reveal stimulus specific properties, which are attributed to excitatory regions of receptive fields only.

Three important implications are briefly mentioned: (1) Direction selectivity of a cell depends in 70% of all neurons on the background status. (2) The recently proposed double-opponent for PHMS neurons might be a special case of a more general receptive field organization already present in the primary visual areas. (3) Finally, global processing seems not to be restricted to hierarchically higher substrates, but occurs even at the level of the primary visual area.

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The majority of neurons in the cat lateral suprasylvian (LS) cortex are directionally selective and respond well to moving stimuli over a wide range of temporal frequencies. These neurons suggest that LS is well suited for analyzing image motion. To examine the role of LS motion perception, we tested the ability of cats to discriminate differences in stimulus direction and speed before and after making bilateral lesions in that region of cortex using ibotenic acid.

Preliminary results have been collected from one animal with histologically confirmed lesions of LS. Several small lesions were made along the posterior lateral bank of the sulcus on both sides, extending from 29 to 10. Within this range about 30% of the medial bank was lesioned, with minor involvement of the lateral bank. Contrast sensitivity for detecting moving gratings was unaffected by the lesions. The cat was also able to discriminate grating direction at thresholds contrasts over a wide range of temporal frequencies (2-10 Hz). However, its ability to discriminate small differences in speed was normal only at low temporal frequencies (2.2 Hz). At higher frequencies, speeds could be discriminated only at high grating contrasts (0.4%). Above 4.5 Hz, speed discrimination could not be measured even at very high contrasts. By manipulating spatial frequency of the gratings, we determined that these deficits in speed discrimination were specific to high temporal frequencies, not to high speeds, i.e. the cat could discriminate speeds even at a relatively high basic speed (16 deg/sec) as long as the gratings were of low temporal frequency (<4 Hz).

These preliminary results suggest that cortex in the lateral suprasylvian sulcus may be involved in the analysis of stimulus speed but not necessarily direction. Thus, direction and speed may be processed independently at later stages of motion analysis. Supported by EY06175 and EY03139.

451.9 Directional selectivity of simple cells in cat striate cortex from linear spatial summation. The Rockefeller University, N.Y., New York.
When stimulated with contrast reversing gratings, any neurally summing directional receptive field (RF) should show continuous variation in response phase as the position of the grating is varied. More quantitatively, it can be shown that the amplitude and phase of the response to drifting gratings would vary with the stimulus phase as a simple functional form, most simply modeled as an ellipse in a polar plot of amplitude and phase. Moving gratings give gratings of the model. Our data from simple cells show an excellent fit to this form, arguing for a high degree of linear spatial summation for non-directional stimuli. Furthermore, we have found by fitting this function, a quantitative prediction of the directional behavior of the model. To test this, gratings of the same spatial frequency, temporal frequency, and contrast as the contrast reversing gratings were drifted in the preferred and non-preferred direction. We have found a very high correlation between the predicted directionality from the contrast-reversing gratings and the response to drifting gratings, often predicting >50% of the actual directionality. Studies parametric in spatial and temporal frequency suggest that the directionality varied with these parameters, often decreasing at higher temporal frequencies, a variation which was mirrored in the prediction from the static gratings.

The linear mechanisms of directional selectivity were robust even in small regions of the RF. First, when the RF was masked except for a narrow window entirely within an "on" or "off" region, the cells still showed directional selectivity to drifting gratings. Even when less than a quarter of a cycle was visible at any time. Second, when static narrow bars (rather than sine-wave gratings) were modulated at low spatial frequency and/or low temporal frequency, the response phase varied continuously across the RF. Studies parametric in spatial and temporal frequency suggest that the response phase change does not seem to be due to simple delays, but to more complex changes in pre- and post-stimulus integration. Therefore, rather than describe the stimulus dependence of on and off regions (even with variable temporal delays), a more complex characterization of the temporal characteristics necessary. In conclusion, we have found that the temporal response characteristics of these simple cells vary continuously along the primary visual axis, this variation explains a good deal of the directionality of these cells.

Supported by NIH T32 GM 07739 and EY 1472.
451.9 AREAS 17 AND 18 CONTRIBUTE THE MAJOR FUNCTIONAL INPUT TO TWO SUPRASYLVIAN CORTICAL AREAS IN THE CAT DURING THE GENERATION OF CYCLOPEAN MOTION (Supported by DFG, Po 121/13-1, project no. 4) - R.J. Tusa and C.B. Smith, Johns Hopkins Hospital, Baltimore MD 21205 and Lab of Cerebral Metabolism, NIMH, Bethesda MD 20892.

In order to better define the cortical regions involved in ovo-tokinetic nystagmus (OKN), 2 techniques have been employed: 1) direct measurement of local rates of glucose utilization during the generation of OKN (autoradiographic experiments of Sokolowski et al. 1977) and 2) ablation of restricted portions of cortex. Four groups of animals were used: normal cats viewing a stationary OKN drum, normal cats generating OKN, cats with a unilateral and bilateral lesion generating OKN, and in one cat with a unilateral and bilateral lesion generating OKN in all 4 cats generating OKN the drum was rotated in one direction, and the eye movement was measured in a plane at an angle to the plane of rotation.

There was an increase in metabolic activity in discrete portions of visually responsive cortex in normal cats generating OKN. These regions coincided with areas 17 and 18 and 4 areas in suprasylvian cortex (21a, 21b, area 19, area 20). A functional reorganization of the suprasylvian areas has been previously shown to decrease OKN gain (slow phase eye velocity/target velocity) to stimuli moving toward the side of the lesion (Neurosci., 9154, 1983). Unilateral ablation of areas 17 and 18 did not decrease OKN gain nor did it affect the metabolic activity in the suprasylvian areas. Unilateral ablation of areas 17 and 18 plus section of the corpus callosum, however, resulted in a decrease in OKN gain toward the side of the lesion. This lesion also decreased the metabolic activity in areas 21a and PMLS ipsilateral to the 17 and 18 ablation but did not decrease the metabolic activity in areas 21b and VLS.

The results suggest that in normal cats generating OKN, the increase in metabolic activity in areas 21a and PMLS depends on an input from areas 17 and 18. At smaller disparities, gratings appeared to move in depth. However, movement in the z plane; at larger disparities, apparent movement occurred in either ipsilaterally or through the corpus callosum. This increase in metabolic activity may reflect sensory information used in the generation of OKN.


A lesion within the central representation of the visual field results in a homonymous field defect in one or both eyes. Because of the particular locus of lesion in one such patient (see also: Poppel, Naturwiss.72 (1985)599; Nature 320 (1986) 523) the question of perceptual completion across a cortical blind spot could be answered. If a long stick is used as a stimulus, the patient is able to perceive the stick in the right upper and right lower quadrant if the stick is kept stationary (2D) as well as if the stick moves the patient reports seeing the entire stick. This stimulus completion appears to be easier with movements towards the fovea. Completion was also observed when a particular point on the stick was moved behind an occluder; thus, no residual visual functions or "blindsight" could be demonstrated. Poppel et al. Nature 243 (1973) 285. If the stick was kept stationary in the upper and by the other eye in the lower quadrant, completion was still possible; thus the effect must be central. If the stimulus had one color throughout completion was easily obtained when the color above and below the scotoma was the same. When the color was different completion was observed when only one color was present. Supported in part by DFG, Po 121/13-1, project no. 4.

(Sponsoring: Poppel)

Recent models of neural motion detection involve establishing a correspondence between local image luminance levels that are displaced in space and time. Previous physiological studies have found substantial support for luminance-based mechanisms at various levels of the visual pathway. These models fail, however, to account for feature elements that are not discriminable by luminance contrast; it is well known that motion can be perceived when viewing patterns that contain moving figures defined solely by isoluminant features such as motion, texture, stereoscopic depth or (under some conditions) chromatic contrast.

We have studied motion sensitive cells of extrastriate visual area MT in an effort to determine whether this neural system mediates isoluminant motion processing. We now report physiological evidence of detected licking for low motion, which exhibit form-cue invariant responses, i.e., responses that are invariant over different forms.

We recorded activity from 49 MT neurons in macaques that were anesthetized with N2O and immobilized. Our stimulus set included:

1) luminant bars that were either brighter or darker than background,
2) isoluminant motion bars defined by random or oscillatory motion of dots within a bar-shaped region, and
3) isoluminant texture bars defined by easily discriminable texture patterns that changed significantly across successive temporal frames.

Stimuli were generated on a video display and moved through the classical receptive field in four directions across a static randomly textured background. Two-thirds of our sample of MT neurons that were selective for the direction of motion of conventional luminant stimuli also responded selectively to motion of one or more of the isoluminant stimuli. Moreover, for 90% of the cells that were selective with multiple stimulus types and found selective for isoluminant motion, both response magnitude and strength of directional tuning were similar for all stimuli.

These findings indicate, first, that isoluminant motion perception may be mediated by a population of MT neurons. Second, they imply that some form processing based upon isoluminant cues precedes motion detection in MT. Recent reports of V1 and V2 neurons sensitive to stimuli defined by stereo depth (Poggio et al., Vision Res., 25:397, 1985) or texture cues (DeUse et al., Nature, 318:61, 1985) are consistent with this view. Third, form-cue invariance suggests a convergence of information from different form channels, either in MT or at some earlier stage. Lastly, these results identify MT with what has been referred to as the long-range motion system; as such, we should expect to find that MT directional selectivity is sensitive to a variety of stimulus configurational and contextual factors.

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Local cerebral glucose utilization (LCGU) was measured in 66 brain regions of 28 awake rhesus monkeys, aged 1 to 24 yr, by using the quantitative, autoradiographic [14C]Deoxyglucose method. Under ketamine hypnosis, animals were restrained in a standard apparatus on the evening before the experiment. On the following morning, they were anesthetized with halothane 1% in oxygen, delivered by face mask. Femoral artery and vein catheters were placed surgically, and the skin incision was infiltrated with lidocaine and closed. Animals were chaired and allowed to regain consciousness in a light- and sound-controlled room. [14C]Deoxyglucose, 80 μ Ci/kg ± 50-55 Ci/mol, was administered intravenously 6 h later, and LCGU was measured during the subsequent 45 min incorporation period. Monkeys were grouped according to age: 1 yr (N=6), 3 yr (6), 9-12 yr (6), 15-18 yr (8), 24 yr (2). For each brain region, the LCGU in each age group was compared to the 3 yr (young adult) mean, by using the Dunnett multiple comparison test.

No significant differences in mean LCGU were found between the age groups and 9-12 yr in any brain region. Significant declines (p < 0.05) were found in only 66 regions in 15-18 yr monkeys (compared to 3 yr); three comparisons may have achieved statistical significance by chance. The areas in which LCGU declined significantly were frontotemporal cortex, area 10, layers IV and V; retrolaminal thalamus, area 8, layer IV; motor cortex, layers III and IV; caudate nucleus; red nucleus; superior olive; inferior olive. LCGU was similar in 15-18 yr and 24 yr groups.
PERFORMANCE OF AGED RHESUS MONKEYS ON A VISUOSPATIAL TASK. 
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The task was presented to the monkeys in a dimly lit room on a platform in a box, which was located in the middle of the room. The box was divided into four equal sections, each of which contained a different color. The monkeys were required to move a red circle on a piece of paper from one section of the box to another section of the box, and then to move it back to the starting section. The task was repeated for a total of four trials, each of which lasted for 120 seconds. The monkeys were considered to have completed the task if they moved the red circle from one section of the box to another section of the box, and then to move it back to the starting section, within the 120-second time limit.

COGNITION OF AGEED RHESUS MONKEYS ON A VISUOSPATIAL TASK. 
M. Brickman*, J. Bachevalier, L. R. Waterman*, L. C. Walker, 
Military Medical Research Laboratories, National Institute of Mental Health, 
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The task was presented to the monkeys in a dimly lit room on a platform in a box, which was located in the middle of the room. The box was divided into four equal sections, each of which contained a different color. The monkeys were required to move a red circle on a piece of paper from one section of the box to another section of the box, and then to move it back to the starting section. The task was repeated for a total of four trials, each of which lasted for 120 seconds. The monkeys were considered to have completed the task if they moved the red circle from one section of the box to another section of the box, and then to move it back to the starting section, within the 120-second time limit.

The measurement of daily patterns of activity provides a non-invasive and unobtrusive behavioral measure of an organism's response to the external environment. We continuously monitored, for four days, patterns of activity of 77 healthy adult humans using a small, wearable, portable activity monitor. The device device counted total number of above-threshold movements of an individual in 15-minute blocks of time and recorded the results in individual monkeys. We found that the monkeys were significantly more active during the night (23:00-07:00 h) periods (p<.005). Consistent with their increased levels of activity, the monkeys showed a significant increase in their circadian rhythms of activity when living in a controlled environment. The results of this study suggest that in nonhuman primates multiple neural systems are vulnerable to increasing age.

Previous studies of regional Cerebral Blood Flow (rCBF) in Alzheimer’s disease (AD) have documented cortical perfusion deficits in long duration AD patients. Accurate differential diagnosis and correlation with neuropathological findings have been demonstrated in these samples (Rinberg and Gustafson, 1985). This laboratory, accurate differential diagnosis has been shown in the early stages of disease progression. The purpose of the current study was to examine the sensitivity of CBF to disease progression in short duration AD patients and to evaluate the relation between functional decline and CBF.

Methods Ten AD patients were referred by a neurologist and given the diagnosis of AD based upon comprehensive examination. Disease progression was quantified by indices of cognitive function (Modified Mini-Mental Status Test,mMMS, means=33.3±1.69; 27.6±17.48, first and second visit, respectively), impairment of activities of daily living (Blessed Dementia Rating Scale,BDRS, mean=8.10±2.68; 11.15±4.87) and estimated disease duration (mean=2.6±1.80; 3.5±1.63). rCBF was measured technologically with 16 detectors over each cerebral hemisphere. The data presented in this report were derived in an electroencephalogram model (Prichonik, et al., 1985) and are presented in terms of the Initial Slope Index (ISI, means=42.14±4.89; 37.07±6.88), a measure representing primarily grey matter flow. Subjects were evaluated on two separate occasions (mean follow-up interval=1.0±0.47). The results in this study were compared with the subject at rest, with eyes closed and ears unoccluded.

Results Repeated measures analysis of variance revealed significant declines in mMMS (F=1.08, p<0.10), BDRS (F=1.78, p<0.01) and global grey matter flow (F=1.0, p<0.1) at follow-up evaluation. Decline in grey matter flow was significantly correlated with decline in mMMS (r=72, p<0.05).

Significant correlations between declines in mMMS and regional flow values were identified in bilateral frontal, parietal and temporal areas. These regions show the greatest degree of deficit when AD patients are compared to age matched normal controls (Prichonik, et al., 1986). Change in BDRS and duration were not significantly correlated with flow. There were no significant intercorrelations within the severity indices.

These results demonstrate characteristic changes in the pattern of cortical perfusion in AD at short-term follow-up and support the sensitivity of CBF to disease progression.

A POSSIBLE EEG BIOMARKER FOR ALZHEIMER’S DISEASE: DOMINANT OCCIPITAL FREQUENCY (DOF)

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DoF (or alpha rhythm) is a predominating rhythmic EEG activity seen in occipital or other scalp regions. DOF is considered to be generated by EPSPs, IPSPs or other membrane voltage fluctuations in underlying neuronal membranes. DOF is known to slow in frequency with normal aging, possible as a result of age reductions in dendritic spine density in Alzheimer’s Disease (AD). Here we report that DOF slows in AD in parallel with the severity of AD. Subject groups were 83 control (CDN), 42 major depressives (DEPXDSMIII), 46 early AD patients (EARLYXPoss), and 25 moderate AD patients (MODAD). These groups were compared to age matched normal controls (Bunt-Milam, et al., 1986).

Change in BDRS and duration were not significantly correlated with flow. There were no significant intercorrelations within the severity indices.

These results demonstrated a characteristic change in the pattern of cortical perfusion in AD at short-term follow-up and support the sensitivity of CBF to disease progression.

DOMINANT OCCIPITAL FREQUENCY (DOF) IN PROBABLE ALZHEIMER’S DISEASE: A POSSIBLE USEFUL BIOMARKER OF EARLY STAGE ALZHEIMER’S DISEASE.

M.V. Vitiello* and P.N. Prinz, Dept. of Psychiatry and Behavioral Sciences, University of Washington and American Lake VAMC, Seattle, Washington 98195. (SPONSOR: A. Bunt-Milam)

Several studies have reported the potential usefulness of quantifications of cortical slowing to serve as a regional marker of Alzheimer’s Disease (AD), all have involved AD patients with significant cognitive impairment. Here we report on the usefulness of Dominant Occipital Frequency (DOF) to distinguish early stage AD patients. 85 control (C) subjects (67.8±7.1 years, 46% male) and 46 AD subjects (70.4±7.5 years, 64% male) were studied. Control subjects were further divided into three groups based on memory complaints; non-complainters (NC, N=28), complainers without validation (NCV, N=24) and complainers whose problem was validated by a significant other (VC, N=32). AD patients were divided by NINCDS criteria into Possible AD (POSS, AD N=16) and Probable AD (PROB AD, N=30) groups. DOF was quantified by 10 or more second frequency counts obtained when activity was most rhythmic, during quiet wakefulness. Figure 1 shows group mean ± SEM for DOF and for a measure of AD severity, the Mini Mental Status Score. The EARLY AD group had significantly lower DOF scores compared to the CON (F=6.41,Lp<0.01) and DEP (F=23.1,Lp<0.001) groups. DOF continued to decline with increasing severity of AD. The data are consistent with the hypothesis that DOF may index related cortical dendritic degeneration, part and parcel of the disease process. Cortical neurites oriented such that their potential fields are detectable in the scalp EEG are more exposed to the cortical surface.

Supported by VA Medical Research and the Dept. of Energy.

Alzheimer’s disease (AD) is a progressive, dementia disorder that compromises a variety of cognitive, sensory, and motor functions. Examiners and clinicians have noted substantial differences between patients in the disease’s symptoms and rate of progression. What is not known is whether individual patients’ rates of change early in the disease are related to their rates of change later on. We therefore studied intra-patient correlation in rate of deterioration among 26 participants throughout an average of 2.31 years; we observed no correlation whatsoever.

All patients were diagnosed by standard procedures as having probable AD; all were seen at least four times in the memory disorders clinic of a general hospital. At each visit, patients received the Blessed Dementia Scale (Blessed, Tomlinson, & Roth, 1968), a standard measure of dementia severity that included subcategories for memory, orientation, and activities of daily living. The scores of all patients were then entered into a computer program that determined by analyses of rate of change (in points per year) from Visits 1 to 2 (mean interval = 2.4 years) and from Visits 3 to 4 (mean interval = 1.7 years). Correlations between rates of change from Visits 1 to 2 with that from Visits 3 to 4 did not approach significance for either Blessed Dementia Scale or Blessed Dementia Scale-Short Form. Therefore, our conclusion, therefore, is highly selective for DAT in otherwise healthy individuals.

Twenty-two of the 43 subjects (51%) developed psychotic symptoms during the course of the illness. Twenty-six percent became psychotic when the illness was in a mild stage. By the time a moderate stage of DAT (CDR2) had been reached, 42% demonstrated psychotic symptoms. In the 26 subjects who reached a severe stage of DAT (CDR3), 54% had developed psychotic symptoms during the course of their illness. Delusions were more severe than hallucinations.

Subjects were evaluated every 6 months throughout the study. When compared to those who remained free of psychotic symptoms and were not lost to follow-up, (n=16) subjects with early psychotic symptoms (n=10) deteriorated more rapidly in terms of cognitive status as assessed by the Blessed Dementia Scale and the Short Portable Mental Status Questionnaire of Pfeiffer. On the other hand, by the end of the 66 month follow-up, 8 of the 10 subjects with early psychosis were still alive versus 6 of the 15 non-psychotic subjects (p<0.05). Therefore, early psychosis in DAT correlates with more rapid cognitive deterioration but not increased mortality.

TRANSMITTERS AND RECEPTORS IN DISEASE V

ACUTE, MASSIVE STRIATAL Dopamine RELEASE IS ASSOCIATED WITH STRIATAL VULNERABILITY TO TRANSIENT MIDDLE Cerebral Artery Occlusion in the Rat. M.T. Glugla, M.D. Giglberg, R. Busato, W.D. Dietrich, R. Hartlemeier, and L. Waldes. Cerebral Vascular Disease Research Center, University of Miami, School of Medicine, Miami, FL 33101.

Neurotransmitter release is believed to play a major role in mediating ischemic damage in select vulnerable brain regions. Small- to medium-sized striatal cells are among neurons known to be selectively susceptible. The striatum, which receives abundant glutamatergic input from the neocortex, is also richly innervated by the nigrostriatal dopaminergic projections. In this study we evaluated the effect of dopamine (DA) depletion on the extracellular release of striatal glutamate (Glu) and DA during ischemia and recirculation, and correlated that with postischemic histopathological changes. Two weeks after unilateral SN lesion, a microdialysis probe was stereotactically implanted into the ipsilateral striatum and subsequently perfused with Ringer solution at a flow rate of 2 µl/min. After a stabilization period of 1.5 h, rats were subjected to 20 min of ischemia by 4-vehicle occlusion combined with systemic hypotension. Samples of the perfusate, representing extracellular fluid, were collected at 10 min intervals, 30 min before ischemia; during ischemia; and during the first 30 min and at the end of 1 h and 2 h of recirculation. The samples were analyzed for DA by RP-LC-EC and for Glu by enzymatic assay. A different group of DA-lesioned rats (n = 10), were evaluated for histology, were perfusion-fixed after 3 days of recirculation, and quantitatively counting of ischemic neurons was performed. Local cerebral blood flow (CBF) fell to 20 ml/100 g tissue/min., DS in addition to preventing severe CBF fall, appeared to be cytoprotective of the cortical ischemic tissue as demonstrated by sparing of catecholamine-containing varicosities in cortex and sparing of catecholamine-containing varicosities in cortex following the MCAO insult.


The biochemical cascade that follows embolic stroke has been causally linked to numerous sub-systems. Among the sub-systems that have been suggested as the triggering cause for the pathophysiology is ischemia after stroke is (a) calcium (b) hydroxy radical formation, (c) prostaglandin changes and (d) opioid receptor activation. Studies have shown that when cerebral blood flow (CBF) falls to 20 ml/100 g tissue/min., neuroelectric activity is significantly reduced in brain. If CBF is further lowered to 12-15 ml, neuroelectric activity may be abolished. A CBF of 6 ml or below generally leads to cellular ionomic imbalance and eventual neuronal death. The duration and extent of reduced CBF may therefore critically determine the clinical outcome of embolic stroke (Astrup, J., J Neurosurg 56:482 (1982); Biive, C., et al, J Cereb Blood Flow Metab 1: (Suppl.) 17:918 (1981). In the present experiment, we investigated the effect of drugs that affect the sub-systems related to calcium, hydroxyl radicals, prostaglandins and opioid receptors to determine whether they could modify an experimentally-induced vascular occlusion in brain. Cats were randomly divided into 6 groups and subjected to middle cerebral artery occlusion (MCAO) via a transorbital approach. After 1 or 4 hours, each group was injected with saline (5); (ii) naloxone (NX) 2 mg/kg, (iii) nimodipine (NM) 1 mg/kg, (iv) protacycin (PGI2) 0.2 mg/kg/ min x 25 min.; (v) dimethyl sulfoxide (DS) 1.5 g/kg, 40% solution. Following these results, an additional group was added to test the outcome of PGI2 + DS. At various time points, local cerebral blood flow (CBF) was measured using micro-electrodes in cortical tissue surrounding the MCAO. Histologic examination and grading of cortical catecholamines was done 24 hours later.

Our results indicate that only PGI2 and DS were able to modify TCF reduction after MCAO. The combination of PGI2 and DS in addition to preventing severe CBF fall, appeared to be cytoprotective of the cortical ischemic tissue as demonstrated by sparing of catecholamine-containing varicosities in cortex following the MCAO insult.

Supported by the Heart and Stroke Foundation of Ontario.
453.3 IN VIVO MEASUREMENTS OF RAT BRAIN Bmax AND KD OF [3H]HEAT, AN α1-ADRENERGIC RECEPTOR ANTAGONIST.
D. Edsall, A. Gledé*, M. Diksic, A. Sherwin*, and A. Nakatomi* (Spon: J. Wolfe). Montreal Neurological Institute and Hospital, Montreal, Quebec, Canada.

In studies in rat brain have shown (125)iodo-2-(beta-(4-hydroxyphenyl)-ethyleniminomethyl tetraol, to be a high affinity α1-adrenergic antagonist, binding to discrete Bmax of brain. The present study aimed to characterize the blood-brain barrier's (BBB) permeability and the steady-state volume of distribution of tritiated HEAT in brain regions.

1) Bmax permeability and volume of distribution: 30-40 μl of drug ([3H]HEAT, 0.20 Ci/mmol) were injected i.v. Rats were decapitated at various times following injection, up to 1 h. Blood samples were taken continuously and measured in triplicate. Protein was measured in triplicate.

2) Kd and Bmax: 38 μl of [3H]HEAT and from 0.5-500 μmol HEAT were injected i.v. and circulated for 1 h. Blood samples were taken at the time of decapitation. Autoradiograms were prepared as above.

RESULTS: 1) BBB transport rate was determined by [3H]HEAT plotted against the normalized plasma time-integral. The BBB permeability (Kp) averaged 0.34±0.04 (SB) ml/g/min. The cerebellar cortex showed no binding and the brain-steady-state volume of distribution (partition coefficient) averaged 2.24±0.01 ml/g. Binding regions included the olfactory bulb, frontal-parietal cortex, thalamus, medial geniculate and pineal gland. In these regions, the equilibrium volume of distribution averaged 5.4%±0.6 (S.E.M.) indicating a Bmax/Kd ratio (receptor potential) of 4.4. 2) The affinity (Kd) and receptor density, Bmax, were calculated by Scatchard analysis (n=6) in various regions. A Scatchard plot representing the uptake of tritiated HEAT in the hippocampus is shown: Bmax=144.4 pmol/g; Kd=27.1 nM.

3) [3H]HEAT crosses the BBB, binding in discrete regions which represent functional alterations.

Earlier studies in rats that have received electroconvulsive shocks (ECS) have shown that the α receptor density increases 24 h after the last ECS. In vivo experiments are planned to evaluate receptor density changes at various times after ECS.

453.4 RAPID REDUCTION OF GERBIL FOREBRAIN ALPHA,-ADRENERGIC RECEPTORS DURING CAROTID OCCLUSION.
K. Schliephake* and J. Divac. Department of Neurology (Neurology) and Pharmacology, Duke Univ., Durham, NC 27705.

In working with brain alpha-1 adrenergic receptor binding, we noticed a reduction in receptors in animals subjected to mild ischemia before sacrifice. In order to characterize this reduction, we subjected Mongolian gerbils (55-70 gms) to 5 or 10 minutes of occlusion under 0.1% halothane and 2.5% isoflurane anesthesia. Membrane homogenates were prepared from frozen forebrains and [3H]-prazosin binding measured at equilibrium using rapid filtration and liquid scintillation counting.

Less [3H]-prazosin binding sites were present in gerbils exposed to 10 minutes of ischemia (57.0 ± 8.1 pmol/mg) compared to controls (117.7 ± 0.2), but the affinity of the binding sites for [3H]-prazosin was unchanged (1.7 nM). Decreases in [3H]-prazosin binding were present after 5 minutes of occlusion, but were maximal after 10 minutes and persisted at 1 hour of reflow.

In another experiment, alpha-1 adrenergic binding was compared to beta adrenergic and muscarinic cholinergic receptor binding in the same membranes. Binding was reduced in membranes from animals after 10 minutes of carotid occlusion compared to controls by 61% ([3H]-prazosin), 25% ([3H]-[2]-cyanoindolindol + ICI 118,551), 33% ([3H]-[2]-cyanoindolindol + ICI 89,406), and 18% ([3H]-QNB). Unexpectedly protein content of the ischemic brain by 20%.

These experiments show that there is a rapid loss of alpha-1 receptor binding in ischemic forebrain and that this loss is greater than that described for some other membrane receptors. Although underlying mechanisms and biological significance of this observation are not clear, we speculate that the change could play an important role in the chain of events that leads to selective neuronal damage after transient ischemia.

(Supported by the V.A. and NS 06233)

453.5 RIGHT CAROTID OCCLUSION ALTERS DITHYDROPYRIDINE CALCIUM ANTAGONIST BINDING IN VARIOUS RAT BRAIN AREAS. M. H. Magnoni*, B. Govaert, F. Battaini**, F. Rossit* and M. Trabucchi***. Institute of Pharmacological Sciences, University of Milan, Istituto Scientifico Sanatrix, Venafro and Chair of Toxicology, 2nd University of Rome, Italy.

Increasing evidence suggests the role of calcium ions in the pathophysiology of the ischemic brain. In particular, the interruption of cerebral blood flow results in a chain of events, sustained by the increased influx of calcium ions in the ischemic cells, that ultimately lead to neuronal necrosis. These major mechanisms allowing the involvement of the extracellular compartment are the opening of voltage operated calcium channels (VOCC). Three different types of VOCC (T, N, L) have been identified in neurons, which differ for electrophysiological and pharmacological features. In particular, L-type is specific to organic calcium antagonists of the dithydropyridine (DHP) class and may be studied at biochemical level using tritiated DHPs. In this line, we have explored the hypothesis that the characteristics of L-type calcium channels, may be modified by ischemia.

Cerebral ischemia was experimentally induced in rats by 1 h ligature of the right carotid. Tritiated PN 20-110 binding was measured in P2 membranes from hippocampus, cortex, striatum, and cerebellum. The hippocampus was the only brain region showing a significant increase of PN binding compared to control (non-occluded). 128 ± 10 f. moles/mg prot. (right, occluded); 129 ± 12 f. moles/mg prot. (left, non-occluded). These increases were present after 5 minutes of occlusion and persisted at 10 minutes of occlusion. The increase of calcium ion influx into the cells in ischemic conditions. It is important to note that the effect is selective, at least at early time, for the hippocampus. This was a particularly vulnerable to ischemic insults and rich in calcium channels. The ischemia-induced modifications of PN 20-110 binding characteristics suggest the involvement of L-type calcium channels in the alterations of neuronal excitability and calcium movements observed during ischemia.


Myasthenia Gravis (MG) patients produce auto-antibodies directed against the nicotinic acetylcholine receptor and other poorly characterized non-cholinergic antigens expressed in skeletal muscle. Thymic hyper trophy and thymus are commonly associated with MG. MG antibodies recognize antigens shared by muscle and thymus (including a putative thymic AcChR) suggesting that thymic antigens may be involved in the pathogenesis of MG.

In order to study the non-cholinergic antigens involved in the MG autoimmune response, we have constructed a cDNA expression library from adult human skeletal muscle. This library was screened with MG sera reactive toward a series of muscle antigens (as determined by Western blotting of human muscle extracts and detection of immunoreactive material in these blots by MG sera). We report the isolation of four muscle-specific clones (1.1 - 1.4 Kb) that do not react with normal human sera but show reactivity toward MG sera lacking anti-AcChR activity or activity against demeasured AcChR subunits.

To investigate the role of thymic antigens in MG we have focused our attention on the well-characterized AcChR. The thymic expression of AcChR transcripts that code for the relative cholinergic antigens has been investigated. Probing Northern blots with RNA transcripts from a subclone, (Internal Pro-1-1ne II fragment) derived from the mouse AcChR, which codes for the AcChR of the mouse cell line NC2B-1 (Boulter, J. et al., J. of Neuroscience, 1991) showed that the RNA species with a length of approximately 3.9 Kb using high stringency conditions. The mRNA species detected by the probe under identical conditions but slightly upregulated and detected at 2.2 Kb. Employing the same probe we isolated from a human thymus library two cDNA clones each one carrying an approximately 3.6 Kb insert. These clones have been sequenced and their muscle counterparts is currently under investigation.
453.7 EFFECTS OF NICOTINE ON PLATELET UPTAKE OF SEROTONIN IN-VIVO AND IN-VITRO. A.A. Kent*, P. Cinciripini*, J. Campbell*, P. Emmens, S. H. McKendall, Dept. of Neurology, Psychiatry, and Pharmacology, Univ. of Texas Medical Branch, Galveston, TX, and (JC) Dept. of Psychiatry, Univ. of Kansas Medical Ctr, Kansas City, Kansas.

Blood platelet uptake of serotonin (5-HT-uptake) has been of interest as a biological marker of neurotransmitter abnormality in psychiatric and neurological disorders. Because of the high incidence of smoking in these patient groups, the effects of nicotine on 5-HT-uptake need to be clarified. Nicotine (NIC) has been shown to enhance the stimulated release of 5HT in animals after chronic administration. We were interested in whether these effects were reflected in human subjects. Three studies were performed: (1) 5-HT-uptake in cigarette smokers versus age-matched controls, (2) 5-HT-uptake in smokers abstinent overnight before and after high (1 mg NIC) or low (0.5 mg NIC) dose cigarette (3) the effects of varying doses of NIC on 5-HT-uptake in-vitro on platelets from non-smokers, abstinent smokers, and sedated smokers. 5-HT-uptake was determined after a 45 minute incubation with 1 X 10^-8 M-SUT (Kent, et al, J AIC Studies, 1985). As in other studies, 5-HT-uptake showed a seasonal variability, decreasing significantly during winter months. Seasonally adjusted 5-HT-uptake was approximately 13% higher in sated smokers (p<.05). However, 5-HT-uptake was significantly lower by 25% in abstinence smokers. Uptake subsequently increased in 11 out of 14 cases 5 minutes after a high dose cigarette, but in only 4 out 7 cases with the low dose cigarette. NIC in-vitro decreased 5HT-uptake dose-dependently (a given EFFECTS OF NICOTINE ON PLATELET UPTAKE OF SEROTONIN DISORDERS. Because of the high incidence of smoking been of interest as a biological marker of neurotransmitter abnormality in psychiatric and neurological disorders, dominate the primate motor-system disorders induced by repeated co-administration of theophylline (50mg/kg), increases the sensitivity to BIC induced seizures in the PC. Together, the proconvulsant effect of BIC and the anticonvulsant effect of ZCL suggests that the PC is one area where adenosine plays an important role in regulation of seizure activity.


Although a great deal of evidence indicates a role for adenosine in the etiology or control of seizures, the neuronal circuitry subserving these actions is unclear. A discrete region-possibly of fundamental importance to epileptogenesis-exists within rat prepiriform cortex (PC) and has been referred to as Area Temporalis Focal injections of bicuculline (BIC) into PC elicit bilateral motor seizures. We find that injections of 2- and 4-mg BIC (ZCL2) (250g) induced seizures because 3H-AMPA radioligand binding, and BMAA (0.5 mM) weakly displaced 3H-KA and 3H-AMPA binding (46% and 56% of controls, respectively). BMAA was approximately 700-fold more effective in displacing 3H-AMPA binding than BMAA, and only 44-fold more effective in displacing 3H-KA binding. These results suggest that both BMAA and BMAA are involved in behavioral and CNS tissue-culture paradigms. Whereas high concentrations of BMAA have little or no significant activity at QA or KA sites, BMAA has extensive interaction with the QA-receptor and may have some minor influence at the KA-receptor site. Thus, low levels of BMAA, in vivo, might initially exert excitotoxic potential through QA-prefering glutamate receptor systems, while higher levels would likely stimulate both QA- and KA-prefering receptor systems. BMAA, a non-dicarboxylic amino acid with delayed convulsant action, is likely to act indirectly via the NMDA-receptor. Supported by NS 18611 and a grant from the Muscular Dystrophy Association.

453.8 EFFECTS OF BMAA AND BOAA ON KAINATE- AND QUISQUALATE-RECEPTOR BINDING IN ADULT MOUSE BRAIN. S.M. Ross and P.S. Spencer, Departments of Neurosciences, University of California, San Diego, La Jolla, CA 92037.

BOAA-induced PSV and behavioral effects of BOAA and BMAA (0.5 mM) weakly displaced 3H-KA and 3H-AMPA binding (46% and 56% of controls, respectively). BMAA was approximately 700-fold more effective in displacing 3H-AMPA binding than BMAA, and only 44-fold more effective in displacing 3H-KA binding. These results suggest that both BMAA and BMAA are involved in behavioral and CNS tissue-culture paradigms. Whereas high concentrations of BMAA have little or no significant activity at QA or KA sites, BMAA has extensive interaction with the QA-receptor and may have some minor influence at the KA-receptor site. Thus, low levels of BMAA, in vivo, might initially exert excitotoxic potential through QA-prefering glutamate receptor systems, while higher levels would likely stimulate both QA- and KA-prefering receptor systems. BMAA, a non-dicarboxylic amino acid with delayed convulsant action, is likely to act indirectly via the NMDA-receptor. Supported by NS 18611 and a grant from the Muscular Dystrophy Association.


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Withdrawal from chronic treatment with neuroleptic agents such as haloperidol (HAL) results in an increased stereotypic behavioral (BB) response to dopamine (DA) agonists termed behavioral hyperresponsivity (BBH). BBH has been utilized as an animal model of a neuroleptic-induced tardive dyskinesia (TD). The relationship between BB and TD has been criticized recently because post-mortem studies of neuroleptic-treated patients not exhibiting TD during their lifetime did not show a similar profile (receptor proliferation without BB) can be observed in the rat.

Compared to saline treated controls, rats chronically treated with HAL (0.75 mg/kg/28 days) exhibited a 71% increase in their SB response to apomorphine (0.75 mg/kg/96 h following withdrawal). The number of striatal spiroperidol binding sites (D-2) was increased in HAL-treated animals without BB. These results demonstrate here that a similar profile (receptor proliferation without BB) can be observed in the rat.


These results demonstrate that DA receptor proliferation can occur without BB and, in this regard, BB is analogous to TD. These results further suggest that mechanisms other than just DA receptor proliferation are involved in BB, and perhaps by extension, TD. Since trihexyphenidyl and anticholinergics have also prevented the development of HAL-induced BB, muscarinic receptor proliferation may represent a compensatory factor.


It is generally accepted from studies of muscle energetics that phophocreatine (PCr) is an energy store available to buffer changes in ATP concentrations (Meyer et al., Am. J. Physiol., 250: C264-C274, 1986). In ischemic or rapidly contracting muscle, PCr concentration falls nearly to zero before ATP decreases. In the hypoxic or tetanic brain, PCr decreases about 50% and then is stable without any change in ATP. A major role for PCr in the brain is believed to be its role in providing an energy buffer to facilitate neurotransmission. This role is of particular interest in any condition in which brain energetics are compromised.

A new method of measuring brain energetics has recently been developed which is based on the 31P-nuclear magnetic resonance (NMR) technique. This method is based on the detection of creatine phosphate (PCr) concentration in vivo. The PCr concentration is determined by measuring the chemical shifts of the PCr and inorganic phosphate (Pi) resonance which are characteristic of the PCr in the brain. The PCr concentration is then determined from the intensity of the PCr resonance.

In this study, we have used this method to measure the PCr concentration in the different brain regions of mice fed an analogue of creatine, N,N-dimethylglycine (DMG) or control. The results show that the PCr concentration in all brain regions is decreased in mice fed DMG. The decrease in PCr concentration is most pronounced in the cerebral cortex.

454.2 AUTORADIOGRAPHIC EVIDENCE FOR AXONAL TRANSPORT OF DEOXYGLUCOSE. Robert F. Ackerman and Michael E. Phelps Department of Radiological Sciences, Division of Nuclear Medicine and Biophysics; and Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

It is generally presumed that the energy requirements of any given brain structure are normally met exclusively by glucose transported locally from capillaries that course through the brain at 50-100 micro liters. However, we have obtained evidence suggesting that either glucose or glucose-6-phosphate can be selectively transported from neuronal cell bodies to their axonal terminal regions. In the first experiment, 14C-deoxyglucose (2DG) (1 uCi in 0.2 u l) was injected directly into the hyper region of the ventral hippocampus of albino rats; the animals were decapitated 5, 24, or 48 hrs later, and their brains processed for contact autoradiography. Near the 2DG injection site, label was concentrated in the stratum granulosum, the stratum pyramidale, and in some cases subiculum. Far from the injection site, label was concentrated in the stratum granulosum and was minimal in the subiculum. In some structures, the microglia are known to receive axons from the neuronal cell bodies labeled by the injection. In animals injected unilaterally, labeling was confined to the ipsilateral side, the contralateral hemisphere serving as a control for uptake of 2DG that diffused from tissue to blood and then rectified to the brain. In some animals, kainic acid (1 nmol), a neuroexcitatory known to inactivate cortical glucose metabolism, was added to the injection; kainic acid increased the intensity of labeling locally and in adjacent regions. In other groups of rats 2DG was injected unilaterally and kainic acid bilaterally. Kainic acid increased the intensity of labeling locally and in adjacent regions. These preliminary results are consistent with the hypothesis that axonal elements are supplied glucose not only exogenously from nearby capillaries, but also endogenously from their own cell bodies. Supported by the Agency for Health Care Policy and Research.
454.5 REGIONAL CEREBRAL BLOOD FLOW DURING CHRONIC HYPO- AND HYPERGLYCEMIA IN RATS. R. M. Bryan and D. A. Pelligrino. Departments of Surgery (Neurosurgery) and Physiology, The M.S. Hershey Medical Center of the Pennsylvania State University, Hershey, PA and Department of Anesthesiology, Michael Reese Hospital and Medical Center, Chicago, IL (60616).

Regional cerebral blood flow (rCBF) was measured during chronic hypoglycemia in rats. The results were compared to those from a previous study (R.M. Bryan et al., Physiological Measurement, 1982). The rCBF of the cerebral cortex, hippocampus, and thalamus was significantly increased during chronic hypoglycemia. The increases in rCBF were greater than those observed during acute hypoglycemia. The increase in rCBF was correlated with changes in blood glucose levels and was not affected by the presence or absence of insulin. The results suggest that chronic hypoglycemia is associated with increased cerebral blood flow, which may be due to increased metabolic demands or enhanced glucose utilization. These findings have implications for the understanding of the pathophysiology of chronic hypoglycemia and may have potential therapeutic implications for the treatment of diabetes mellitus.

454.4 EFFECTS OF CHRONIC NICOTINE ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT. R. J. Fanelli and E. D. London. Neuropharm. Lab., NIDA Addiction Research Center, Shady Grove, Bethesda, MD (22224).

Previous work from our laboratory has shown that nicotine (N) acutely stimulates local cerebral glucose utilization (LCG) selectively in brain regions with high densities of specific nicotinic cholinergic binding sites. Because N is self-administered chronically, it is important to know its effects on brain function. To this end, we administered the 2-deoxy-D-[1-14C]glucose (DG) technique of Sokoloff et al. (J. Neurochem. 29:897, 1977) to rats treated chronically with N. In a completely random 2 X 2 factorial design, rats received s.c. injections of N tarte (1.0 mg/kg, 0.7 sec) or saline and sacrificed on one of two days (0000 and 1700) for 10 days. They were observed for the presence of selected behaviors (e.g., cranium, ataxia, hyperactivity) at 5, 15 and 30 min after treatment. In the second group, rats were given the same treatment as on previous days, and then were prepared with microdialysis probes for the next 3 days. Following a 3 h recovery period, they received either 0.3 mg/kg of N or saline s.c. (2 min before DG [100 μCi/kg, 1.0V]). Blood pressure, pulse rate, and body temperature were measured periodically during the DG experiments. Animals were killed 45 min after the DG injection and LCG was determined autoradiographically as described by Sokoloff et al. (J. Neurochem. 30:1977). The duration of some of the N-induced behaviors decreased over the 10 days of drug treatment, but behavioral tolerance was not clearly indicated. The frequency and severity of ataxia actually increased with repeated N exposure. Animals given N for the first time on the DG day did not show the same behavioral similarity to that which we observed previously with acute N treatment. Where significant rCBF changes were seen in the interventricular, thalamus, fascicular and substantia nigra pars compacta, and interpeduncular nucleus. Following chronic N, some brain regions (interpeduncular, nigra pars compacta) showed evidence of tolerance in the LCG response to N challenge on the day of the DG experiment. Several brain regions that showed no treatment-related changes in response to acute (nigra compacta) or chronic hypoglycemia had LCGU values lower than the group treated chronically with N but given NaCl on the day of the DG experiment, several brain regions (interpeduncular nucleus, superior colliculus) had LCGU values lower than the group given NaCl alone, which may be related to withdrawal. These findings provide important information regarding the local effects of chronic N and may have implications for behavioral tolerance despite nicotinic receptor regulation in the brain.
454.7 EFFECTS OF COMPRESSION CONCUSSION ON BRAIN MITOCHONDRIAL BIONERGETICS IN THE RAT

L.M. Maher*, J.W. Phillips and P.L. Paterson. Depts. of Neurology and Physiology, Wayne State University School of Medicine, Detroit MI 48201

Closed head injury (CHI) is a major cause of morbility and mortality in man. In animal models of CHI, the most appropriate methods of treatment depends upon a complete understanding of the underlying pathology of the injury. We attempted to clarify the effects of CHI and ischemic reperfusion on cerebral bionergetics in the rat.

Male Sprague-Dawley rats were anesthetized, a craniotomy was done, and body temperature was controlled at 37°C using a heating pad and a temperature controller. Compression-induced concussion was administered with a General Electric Corporation Picoscope using nitrogen pulses at controlled pressures and times. At various time periods (0, 1, 2, and 3 hours) post-injury the animals were sacrificed and brain mitochondria were isolated according to a modification of the method described by Fiskum, 1984. Rates of oxidative-phosphorylation for pyruvate + malate and succinate + rotenone were determined polarographically using a Clark oxygen electrode and compared with tandem control rat brain mitochondria.

Our results revealed a 60-70% depression of respiratory control indices for both mitochondrial substrates only in the 3 hour post-concussion rat, indicating a significant degree of uncoupling occurred. These preliminary results show that it may be possible to initiate a specific treatment regimen prior to the onset of motor dysfunction.

454.8 ANOXIA AND CNS WHITE MATTER: IN VITRO STUDIES USING THE RAT OPTIC NERVE

P. Davis* and B.S. Ransom. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA

The cellular consequences of anoxia have been best studied, to date, in grey matter. We analyzed the effects of anoxia on mammalian CNS white matter, in adults and during development using the isolated rat optic nerve (RON). The compound action potential (CAP) was used as a monitor of RON excitability to quantify the functional recovery of anoxia or of oxygenation. Anoxia for different ages (1 to 90 days) was dissected free, placed in a recording chamber, and bathed at 30°C with a bicarbonate buffered saline solution containing 5 mM [K+].

In the adult RON, CAP amplitude fell to 0 within 2-3 minutes of anoxia onset. CAP recovery was 80-90% of control period of anoxia; in most instances maximum recovery was achieved within 30 minutes. CAP recovery was a linear function of the anoxic interval. Periods of anoxia less than 15 minutes were followed by 80-100% recovery. Anoxia of 90 minutes by an average of 34% recovery, and only after 75 minutes of anoxia was recovery absent. RONs from animals less than 7 days of age were resistant to anoxia; CAP amplitude did not change with anoxia (up to 90 minutes). Sensitivity to anoxia, measured as incomplete recovery from a standard 60 minute anoxic insult, developed gradually from postnatal day 10 to 20 and remained constant thereafter.

Using a standard 60 minute anoxic insult, adult RONs were examined for factors which could influence degree of functional recovery. When the glucose concentration was increased from 10 mM, which is standard for many physiological solutions, to 20 mM, CAP amplitude fell more slowly, and maximum CAP recovery increased from a mean of 31 ± 15% (P < 0.05) and intracellular pH (pHi) were monitored in the RON using ton-sensitive microelectrodes. These studies revealed that the higher glucose concentration was associated with a delayed and reduced rise in [K+], as well as an enhanced acid shift. Enhanced anaerobic energy metabolism, with greater lactic acid production, may explain these changes. The delayed rise in [K+] may account for the delayed loss of excitability seen with 20 mM glucose. Reducing [Ca++] from 2 to 0 mM in anoxic media increased recovery from 31 ± 15% to 86 ± 4% (P < .001). Exposure for 5 minutes to 100 mM glutamate, an endogenous excitotoxin, had no effect on CAP amplitude during or after the exposure.

These studies have begun to define the characteristics of anoxic pathophysiology in mammalian white matter. This tissue is relatively resistant to anoxia, compared to grey matter, may be particularly protected from anoxic injuries by extracellular glucose concentration, and appears critically dependent upon [Ca++] with regard to the degree of irreversible anoxic injury. Supported by Nih grant NS 15869.


454.9 BRAIN EDEMA AND FUNCTIONAL RECOVERY FOLLOWING FOCAL ISCHEMIA IN THE RAT. Teiji Tominaga*, Mitsue Tomimaga*, Chika Katagi* and S. Togoshi. (Department of Neurosurgery, University City Science Center, Phila., PA 19104)

In order to study the relationship between ischemic brain edema and functional recovery, ionic movement, water content and EEG activity were investigated using a rat focal ischemia model. Chlorpromazine (CPZ) was reported to have a beneficial effect on tissue ischemia. Therefore, we studied the effect of this compound. Using Sprague-Dawley rats, the focal ischemic lesion in the territory of the right middle cerebral artery (MCA) was produced according to Chen et al. in 1986. The sham control group served as a pre-ischemic condition. On the 1st, 3rd or 7th day after operation, animals were sacrificed and water content was measured by the wet-dry weight method. Then, sodium, potassium and calcium contents were determined by atomic absorption (Young, et al., CNS Trauma 3:215, 1986). EEG was recorded before and one hour after MCA occlusion under artificial ventilation. On the 3rd and 7th day, EEG was recorded from the same animal in a similar manner and analyzed by Fourier transform. Water content in non-treated and CPZ-treated groups is shown in the Table. Significant increases were obtained in the ipsilateral hemisphere concurrent with accumulation of sodium. Calcium content was increased (Right/Left ratio was 1.40 in Day 3). CPZ suppressed the increase in water and electrolyte movement. In EEG analysis, the power ratio between pre-ischemia and post-ischemia was improved in the treated group. The results indicate that CPZ blocker had a protective effect against brain edema.

Table: Water content (weight %) in non-treated and CPZ-treated groups

<table>
<thead>
<tr>
<th></th>
<th>Non-treated group</th>
<th>CPZ-treated group</th>
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<tbody>
<tr>
<td>Day 1</td>
<td>24.3 ± 0.14</td>
<td>24.0 ± 0.16</td>
</tr>
<tr>
<td>Day 3</td>
<td>28.4 ± 0.14</td>
<td>27.5 ± 0.16</td>
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<tr>
<td>Day 7</td>
<td>31.2 ± 0.14</td>
<td>30.3 ± 0.16</td>
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*Significantly different from sham or non-treated group (P <0.05).

454.10 INVOLVEMENT OF PLATELET-ACTIVATING FACTOR IN CENTRAL NERVOUS SYSTEM DISORDERS. F. Clostre, C. Millarici, C. Beth and P. Brouet

I.H.S. Research Labs, 17 avenue Descartes F-69205 Le Plessis-Robinson (France) (Supported by the European Economic Community). A potential role for Platelet-Activating Factor (PAF) in the central nervous system was suggested by the discovery that Ginkgo biloba (Ginkgo biloba) and some benzodiazepines, antagonize PAF (rev. in Ennifar et al. Pharmac. Rev. 1985). PAF or some of its phospholipids may be involved in cell-to-cell interactions in nature brain, one observation supporting this suggestion being the lack of enzymes in Zellweger syndrom. Alprazolam is used as an antidepressant agent particularly in the treatment of panic disorders. Ginkgo biloba extract (GBE 761) is used clinically for prevention of stroke and psycho-behavioural problems of senescence. Potent and specific PAF-antagonists have been found in Ginkgo biloba (Braquet, Lancet, 1985 ; 1: 1501) termed Ginkgolides (G). The range of activity of these antagonists on PAF-binding assay is the following: BN 52021 (G0) > BN 52022 (G1) > BN 52024 (GC) > BN 52024 (G0). We therefore undertook the investigation of the role of Ginkgolides in several basic syndromes. Alprazolam does not modify the behavioural pattern (open field, tetrazolxine test, electroconvulsive shock) and does not induce myorelaxation. In contrast, in the despair test in the mouse, BN 52021 (1-20 mg/kg p.o.), even at the lower dose significantly reduced the immobility time of the animals [control : 154.7 ± 12.1 sec ; 10 mg/kg : 157.1 ± 10.0 sec ; 20 mg/kg : 170.5 ± 4.0 sec ; 40 mg/kg : 196.0 ± 6.0 sec]. When the glucose concentration was increased from 10 mM, which may be expected to increase (Up to 90 minutes) was recovery absent. RONs from animals less than 7 days of age were resistant to anoxia; CAP amplitude did not change with anoxia (up to 90 minutes). Sensitivity to anoxia, measured as incomplete recovery from a standard 60 minute anoxic insult, developed gradually from postnatal day 10 to 20 and remained constant thereafter.

Using a standard 60 minute anoxic insult, adult RONs were examined for factors which could influence degree of functional recovery. When the glucose concentration was increased from 10 mM, which is standard for many physiological solutions, to 20 mM, CAP amplitude fell more slowly, and maximum CAP recovery increased from a mean of 31 ± 15% (P < 0.05) and intracellular pH (pHi) were monitored in the RON using ton-sensitive microelectrodes. These studies revealed that the higher glucose concentration was associated with a delayed and reduced rise in [K+], as well as an enhanced acid shift. Enhanced anaerobic energy metabolism, with greater lactic acid production, may explain these changes. The delayed rise in [K+] may account for the delayed loss of excitability seen with 20 mM glucose. Reducing [Ca++] from 2 to 0 mM in anoxic media increased recovery from 31 ± 15% to 86 ± 4% (P < .001). Exposure for 5 minutes to 100 mM glutamate, an endogenous excitotoxin, had no effect on CAP amplitude during or after the exposure.

These studies have begun to define the characteristics of anoxic pathophysiology in mammalian white matter. This tissue is relatively resistant to anoxia, compared to grey matter, may be particularly protected from anoxic injuries by extracellular glucose concentration, and appears critically dependent upon [Ca++] with regard to the degree of irreversible anoxic injury. Supported by Nih grant NS 15869.
455.1 A PC-12 CELL SURFACE ANTIGEN WHICH INVOLVES OR REGULATES TRANSMITTER RELEASE. Y. Kuroda, K. Kobayashi and Y. Ghashchi, Dept. of Neurochem., Tokyo Metropolitan Inst. for Neurosci., Fuchu-shi, Tokyo 183, Japan.

A library of monoclonal antibodies against cell surface antigens of rat pheochromocytoma PC-12 cells was obtained. Splenocytes of mice injected with intact PC-12 cells were fused with myeloma. Culture fluid of the resulting hybridomas was first screened by enzyme-linked immunosorbent assay (ELISA). ELISA-positive cells were cloned and the monoclonal antibodies were purified from the serum-free medium. More than 70% of the monoclonal antibodies recognized the cell surface antigens. The monoclonal antibody library was then, applied to in vitro formed cholinergic synapses between cultured neurons to see their effect on synaptic transmission. Among the antibodies added, only one monoclonal antibody (PCH 32-20) blocked the synaptic transmission.

The monoclonal antibody was examined to see its effect on in vivo synapses. Rat superior cervical ganglion was dissected out. The pre-ganglionic fiber was electrically stimulated and the evoked EPSP was observed by a microelectrode in post-ganglionic neurons. The monoclonal antibody solution of PCH 32-20 decreased the amplitude of EPSP and the mean quantal content in a dose dependent manner. This monoclonal antibody stained a broad band around 24K daltons in SDS-PAGE western blot of PC-12 cell membrane preparation. Those data suggest that the monoclonal antibody (PCH 32-20) binds to a antigen on presynaptic membrane of the ganglionic synapse and change its function, for transmitter release itself or for its regulation.

This work was supported by a Grant-in-Aid for Special Project Research on Mechanism of Bioelectrical Response (60115008) from the Japanese Ministry of Education, Science and Culture.

455.2 PURIFICATION AND CHARACTERIZATION OF AN ANTIGEN THAT IS SPATIALLY SEGREGATED IN THE PRIMARY OLFACTORY PROJECTION. J.E. Schoeb and D.T. Settlebe, Dept. Anat. and Neurobiol., Washington University School of Medicine, St. Louis, MO 63110.

The monoclonal antibody designated RB-8 reacts with RB-8 neuropil antigens derived from the ventrolateral olfactory epithelium and their terminals in glomeruli of the ventrolateral olfactory bulb, but not the axons and terminals from the dorsomedial epithelium untrained or lightly stained (J. Neurosci. 6:3393). In the olfactory nerve RB-8 recognizes a 125 kDa, membrane-associated protein. RB-8 also reacts with a 125 kDa membrane protein elsewhere in the CNS and PNS. This antigen is nervous system specific and is found on some neurons but not others. We report here the purification of the 125 kDa RB-8 antigen and its further characterization as a cell surface glycoprotein.

To show that the RB-8 antigen is exposed on the cell surface, explants of the fetal olfactory epithelium in tissue culture were exposed to the RB-8 antibody while living, which results in labeling of the olfactory axons derived from the explants. The staining of living axons with RB-8 antibody is due to the surface localization of the antigen rather than disruption of the plasma membrane, since antibody to visinin, a known internal antigen, does not label axons in parallel control cultures that are fixed and permethylized.

On the assumption that the antigen in brain is the same as the protein in the olfactory nerve, we used whole brain as the starting material for the purification of the RB-8 antigen. The antigen can be purified to homogeneity as follows: (1) the RB-8 antigen is extracted from brain membranes with deoxycholate. (2) The solubilized antigen is highly enriched by chromatography over an RB-8 antibody column. (3) The RB-8 antigen isolated from the other proteins in the column eluate by preparative one dimensional SDS-PAGE and then eluted from the 125 kDa band on the gel. The 125 kDa band in the column eluate is composed solely of immunoreactive protein as shown by immunoblotting of two-dimensional gels. The purified protein was shown to be a glycoprotein by its reactivity with lectin probes. In addition, the purified antigen was used to generate a polyclonal rabbit antiserum that recognizes only the 125 kDa protein on immunoblots. Immunochromatographic staining of the olfactory bulb with this antisera exactly replicates the staining seen with the monoclonal RB-8 antibody. Thus, the 125 kDa protein from the whole brain shares strong immunological homology with the olfactory nerve protein. This additional characterization of the RB-8 antigen and the availability of a polyclonal antisera may prove useful in assessing the functional role of the antigen in the primary olfactory projection. Supported by NIH, NS 08076 and NS 12687.
455.3 A SYNAPE-SPECIFIC CARBOHYDRATE AT THE NEUROMUSCULAR JUNCTION IS ASSOCIATED WITH BOTH ACETYLCHOLINESTERASE AND A GLYCOPROTEIN. S.T. Scott* and J.B. Sanes, Department of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

We previously showed that the lectin DIB (Colchus biflorus agglutinin) selectively stains neuromuscular junctions in rat skeletal muscle and thus defines a synapse-specific carbohydrate (Sanes and Cheney, Nature 300: 646, 1982). The existence of this carbohydrate and the relative simplicity of the neuromuscular junction encourage use of this system to study the involvement of carbohydrates in synapse formation and function. Using a panel of lectins, antibodies, and glycosidases of known specificities as histochemical probes, we found that the carbohydrate selectively concentrated at the neuromuscular junction resides in s-acetylgalactosamine (GalNAc) linked in the J americ form to the termini of olygosaccharides. We also found that a second lectin, VVA-B4 (Vicia villosa B4-lectoglutinin) stains synapses as selectively as, but more intensely than, DIB. Using VVA-B4, we asked whether a similar carbohydrate is concentrated at skeletal neuromuscular junctions in other vertebrates. In fact, VVA-B4 selectively stains neuromuscular junctions in most major vertebrate classes (12 species), a phylogenetic conservatism that suggests a synapse-related role for GalNAc B-terminal saccharides.

Further study of the synapse-specific carbohydrate requires identification of the molecules to which it is attached. We previously showed that DIB binds the asymmetric, collagen-tailed form of acetylcholinesterase (which are highly concentrated at the rat neuromuscular junction) but not globular forms (which are more widely distributed) Scott and Sanes, Neuron. Abstr. 10: 546, 1984). VVA-B4 also binds asymmetric but not globular acetylcholinesterase. We have now identified a second GalNAc-bearing glycoconjugate immunohistochemically, using a monoclonal antibody, anti-SEA-3, which recognizes GalNAc-bearing lipids (Shavinsky et al., Cell 30: 697, 1982) anti-SEA-3 stains neuromuscular junctions selectively. EMSA-immunoreactive material is glycoprotein-like (choloroform/methanol extractable) and distinguishable from AChE, but staining is blocked by VVA-B4. One of two antisera to globoside (a GaINAc terminal galactoside) tested also stains synapses selectively. The association of a synapse-specific carbohydrate with at least two different synapse-specific molecules raises the possibility that the former plays a role in determining a property that the latter share, such as their concentration at the synapse. (Supported by NIH and MDA.)

455.4 IMMUNOCYTOCHEMICAL STUDIES OF CELL SURFACE MOLECULES OF MIGRATING NEURONS IN VITRO. W.A. Gregory, C.A. Mason and M.E. Hatten, Dept. of Pharmacology, New York, NY 10016.

In previous studies, we have used high resolution video microscopy to study the movement and cytolgy of cerebellar granule cells migrating along astroglial processes in a microculture system. Correlated electron and video microscopy of actively migrating cells has shown specialized junctions (termed interstitial densities) formed between glia and the annular microtubules. These interstitial junctions are located at the neuronal soma–glial apposition and are characterized by filaments spanning between apposed membranes. These interstitial filaments appear to be continuous with or transmembrane extensions of submembranous cytoskeletal filaments. Extracellular space (20nm) is dilated at these regions. Both microtubules and microfilaments are seen in the leading process, arrays projecting to the plasma membrane. Numerous coated vesicles are seen in the leading process of migrating cells suggesting possible endocytosis of cell surface ligands. Non-migrating granule cells form another type of junction, puncta adherentia, with astroglia.

The present experiments use immunocytochemical techniques at the EM level to define the distribution of cell surface ligands, molecules thought to mediate cell-cell and cell-substrate adhesion. Cultures are being stained with polyclonal antibodies to astroactinin, a molecule involved in granule neuron-glial cell contacts, NILE (LI, NgCAM), B5-2 (IN-CAM), laminin and a mammalian form of integrin, a cell surface receptor for both fibronectin and laminin. Primary antibodies are observed on lightly fixed cultures using FITC-labeled secondary antibody fluorescence or colloidal gold-labeled secondary antibody preembedding electron microscopic techniques. (Supported by grants NS 15439 and 21457.)


Two families of adhesive ligands, cell-cell and cell-substratum, have been proposed to mediate cell contact interactions and cell spreading on culture surfaces, respectively. A question that has not been addressed is how cells choose between cell and/or substrate molecules. Here we have explored the behavior of astrocytoma cells given choices between granule cell substrates and neurons.

To investigate the initial cell-substratum interactions, purified granule cells were mixed with astrocyta and cell surface membrane specializations were monitored with high resolution, time-lapse, video-enhanced differential interference microscopy. When the human U-237 or mouse GI6-24 astrocytoma cells are cultured on untreated glass cover slips, in essence depriving them of an adhesive ligand, they display extensive surface membrane activity characterized by the rapid protrusion and retraction of membrane blebs. These cells fail to settle to the glass surface, even after several hours. Treating the culture surface with either fibronectin or polylysine (100mg/ml) abolishes this blebbing activity and the cells rapidly spread. The addition of neurons to the astrocytoma cells causes a short period of blebbing which ends when cell-cell contacts are established with the granule cell; thereafter the astroglial cell attaches to the culture surface.

When astrocytoma cells are plated with neurons in the presence of the polyclonal antibody anti–astroactinin, which blocks neuron-glial interactions or the monoclonal antibody 1A3, which also blocks neuron-glial interactions, little blebbing is seen on the astrocytoma cell surface and the cells rapidly spread onto the culture surface. Similarly adding the monoclonal antibody DIB, believed to recognize the fibronectin receptor, reduced astrocytoma cell blebbing is seen until neuronal-glial contact is made. These experiments suggest that blocking either neuron–glial or glial-substrate adhesion promotes the alternative choice, thereby reducing the period of membrane blebbing.

These findings, that the exaggerated membrane activity we have observed in astrocytoma cells is involved in cell decision making events, and these choices are related to the availability of cell surface ligands such as fibronectin and integrin-like cell surface receptors for fibronectin and/or laminin.

Supported by NIH grant NS 21097 (M.E.H.)
455.7

**DISTRIBUTION OF LAMININ AND INTEGRIN ALONG TRUNK SATURDAY AM CELL SURFACE MACROMOLECULES II 1637**

(Supported by NSF Grant BNS 86-07760).

In vivo the Jones antigen is present on cells and in the optic fiber layer of the developing retina. In vitro, this antigen's expression on cell soma and neurites of retinal explants from E16-E21 embryos appeared to be modulated by the substrate on which they grew. Explants grown on poly-L-lysine or laminin exhibited Jones staining. Jones expression was high on non-collagen and collagen did not.

One of the natural substrates of retinal ganglion cell axons is optic nerve glia. The Jones antigen, apart from a brief appearance in the optic stalk at E13, is immunocytochemically and biochemically absent from the optic nerve. However, in dissociated P7-P14 optic nerve glia, we found that ~20% of the cells expressed the antigen within one day of and for at least 4 days of culture on poly-L-lysine. Most of the Jones positive glia also expressed galactocerebroside.

To test the hypothesis that the presence of retinal fibers actually inhibits Jones expression by these glia, we co-cultured, on poly-L-lysine, freshly dissociated P14 optic nerve glia with P7-E15 retinal explants. While the retinal fibers continued to express the Jones antigen, the optic nerve glia within 400μm of the explants' edges failed to express the same antigen. Such glia were not necessarily in contact with neurites. In contrast, ~10% of the glia cultured at distances >400μm from the explants, cultured on the same coverslip, were still able to express these antigens.

Our results indicate that the expression of Jones gangliosides is continuously modulated by a complex of interactions between neurites and their substrates and, furthermore, that only part of such interactions are contact mediated.

Supported by Biomedical Research Support Grant RO7015 to Yale University, NIH Grant HD22498 to MCP and a Miles Fellowship to DBK.

455.9

**DISTRIBUTION OF LAMININ AND INTEGRIN ALONG TRUNK NEURAL CRESCENT LABS. Daniela Krotoski and Marianne Bronner-Fraser, Developmental Biology Center, University of California, Irvine, CA 92717.

Interactions between neural crest cells and the extracellular matrix have been proposed to play an important role both in cell guidance and cell differentiation. In avians, neural crest cells have been shown to migrate actively on fibronectin (FN) and laminin (LM) substrates in order to define the neural crest migration when injected in vivo (Bronner-Fraser, Dev. Biol. 117:512-28 1986).

In vivo, interactions between growing neurites and their substrates have been shown to affect the organization and development of the neural tube. Interactions between the neural tube, notochord, somites, dorsal aorta, cranial neural crest and other structures during neural crest migration when injected in vivo (Bronner-Fraser, Dev. Biol. 117:512-28 1986).

Here, we describe the distribution of laminin and integrin in neural crest migration in this species. Xenopus embryos are easily manipulable and, therefore, provide a convenient model for the analysis of vertebrate neural patterning.

We have recently described neural crest pathways in Xenopus laevis using two new cell marking techniques and have also described the distribution of fibronectin along these pathways.

Laminin immunoreactivity was observed along neural crest pathways. In the transverse plane, LM staining was visible in the basal lamina around the neural tube, notochord, somites, dorsal aorta, epidermis, dorsal mesentery and lateral plate mesoderm. This distribution was similar to that of FN, but appeared more fibrous and diffuse than FN. In situ hybridization studies have also been used for the neural tube, notochord, somites, dorsal mesentery and epidermal cells. Additionally, integrin immunoreactivity was detected on the surface of cells located along neural crest pathways along the dorsal neural tube, between the neural tube and somites as well as in the dorsal FN matrix. The expression of integrin appeared to increase with development. In the longitudinal plane, neural crest cells exhibited a periodicity in their migration pattern, migrating primarily along the posterior portion of each somite. In contrast, integrin, LM and FN immunoreactivity appeared uniformly distributed along the notochord and somites prior to, during and after neural crest migration at the light microscopic level. Though these molecules may play a permissive role, they did not exhibit the periodicity required to play a guiding role for neural crest migration.

(Supported by NSF Grant BNS 86-07760.)

455.10


The lym-11 antigen is believed to be the beta 2 microglobulin component of the class I (K and D) histocompatibility antigens. However, it is coded for by a gene on mouse chromosome 2 (Immunogenet, 11:441, 1980), in contrast to the other components of the class I antigens which are coded for by genes on chromosome 17. Allelic forms of lym-11 exist, and can be used as antigens in cell sorting of otherwise identical class I type.

There is an antibody which recognizes the lym-11 allele on lymphocytes of B10.S but not B10.R mice. Previous studies have focused on the tissue distribution of lym-11, by attempting to diminish its activity in a cytotoxicity assay after absorption and lack of activity with different tissues. By this technique, brain did not reduce cytotoxicity, and it was concluded that lym-11 was minimally expressed on brain. In the present analyses, we measured direct labeling of lym-11 on the surface of cultured brain endothelial cells using a cell sorter. This was done before and after exposure to interferon, which are known to affect histocompatibility antigen expression. In addition we studied lym-11 expression on K and D regions by using sections of brain. We demonstrate that B10.S endothelial cells in culture and in brain express this antigen but it is not present on B10.R cells, and B10.S cells also show a lym-11 staining pattern in the brain. Furthermore, the levels of expression drastically rise after several days of exposure to interferon. Since both B10.S and B10.R mice share the H-2b class I haplotype at the K and D regions, this difference in lym-11 expression allows distinction between cells originating from these two strains of mice. This strain distribution thus allows differentiation of bone marrow derived endothelial cells, and will serve to identify these cells in allogeneic transplant studies and in a brain tissue issue between these two different strains of mice.
CARDIOVASCULAR RESEARCH

RESPIRATORY AFFERENT PROCESSING IN THE NUCLEUS OF THE TRACTUS SOLITARIUS IN RATS. D.R. McCormick, A.C. Boshom and D.O. Nelson Departments of Physiology, and Anesthesia, Northwestern University, Chicago, IL 60611.

Pulmonary afferents are known to project to the nucleus of the tractus solitarius (NTS). We have previously shown (Fed. Proc. 46:1420, 1987) that injections of volumes of excitatory amino acids into the NTS, either by microinjection into the acute tractus solitarius, produce site-specific alterations in respiratory motor output and airway function, which resemble the responses to activation of pulmonary and baroreceptor afferents. These studies have been extended to characterize the neuronal activity in the regions from which the respiratory responses were elicited. Experiments were performed in urethane-anesthetized rats in which the diaphragm electromyogram (EMG) or phrenic nerve activity were recorded. End-tidal CO2, arterial pressure and heart rate were continuously monitored.

In some experiments rats were paralyzed with gallamine and mechanically ventilated. Single- or multi-barrel pipettes were used for unit recording and pressure ejection of excitatory amino acids and dyes for marking recording locations. Injection of 1 to 6 nl of DL-homocysteine (DLH, 10 mM, pH 7.4) into the ventral aspect of the nucleus of the tractus solitarius 200 μm caudal to the obex produced a transient surge averaging 150±4% in 15 animals. Recordings of single unit activity in this region, with the same electrode from which DLH was injected, identified neurons which discharged action potentials in phase with diaphragm activity in spontaneously breathing rats. To determine whether these neurons received pulmonary stretch receptor input, lung inflation was manipulated in paralyzed, mechanically ventilated animals. In these studies, DLH injection still produced transient periods of phrenic nerve silence. In other proximal sites in which respiratory unit activity was recorded, discharge to phrenic nerve activity and DLH injection did not silence the respiratory neurons. Therefore, although the number of neurons studied is still small, it would appear from these data that other descending inputs must be important for the control of the diaphragm during breathing.

Supported by grants from NIH (NS02065), NSF (BBS317651), and NASA (NSG2380).

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Supported by grants from NIH (NS02065), NSF (BBS317651), and NASA (NSG2380).
456.5 INSPIRATORY NEURONS THAT ARE ACTIVATED WHEN INSPIRATION IS INHIBITED BEHAVIOURALLY. John Orem, School of Medicine, Texas Tech University Health Science Center, Lubbock TX 79430.

Respiration can be automatic or controlled behaviorally. Behavioral control in the cat occurs, at least in part, through control of the brainstem respiratory neurons that constitute the automatic system. Thus when inspiration is inhibited in response to a conditioned stimulus, inspiratory neurons in the medulla are inactivated. The latency of this inactivation ranged from approximately 40 to 120 ms and averaged 65 ms (±7.5 s.d.) across a sample of 28 inspiratory neurons recorded in the dorsal and ventral medullary respiratory groups. These inactivated inspiratory cells had strong and consistent respiratory signals during spontaneous breathing (mean n = 5 ± 0.51 s.d., a.d., see Orem, J. and T. Dike. J. Neurophysiol. 50:1098, 1983) for an explanation of the n Statistic). In contrast to these inspiratory cells, six inspiratory cells and one expiratory-inspiratory cell were activated (mean latency = 36 ms ± 2.3 s.d.). Inhibition of the inspiratory and expiratory-inspiratory cells located in both the dorsal and ventral respiratory groups were recorded, 6 of 7 cases, in the vicinity of inspiratory neurons that were inhibited during the behavioral response. These activated cells had low %-valued respiratory activity—averaging only 0.27 across cells with a range of 0.03 to 0.60. Thus they receive considerable amounts of nonrespiratory input. It is proposed that these cells act as the interface through which behavioral inhibition of inspiration occurs. Supported by NHLBI Grant 21257.

456.6 PHRENIC INSPIRATORY ACTIVITY MODULATED BY STIMULATION OF PHRENIC SENSORY NEURON CORTEX. F.J. Thompson, F.D. Davenport, J.L. Warner, Dept. of Neuroscience, School of Medicine, College of Med. and Vet. Med., Univ. of Florida, Gainesville, Florida 32610

We recently reported that stimulation of phrenic afferents evoked activity in the sensori-motor cortex in cats and proposed that these inputs are sensory kinesthetic (Davenport, Thompson, Reno, and Freed, 1980). The purpose of the present study was to determine if these cortical areas represent a low threshold region for control of ventilation.

These experiments were done on vagotomized adult cats anesthetized with alpha chloralose (70 mg/kg). The contralateral vagus nerve or motor cortex was stimulated by analysis of sensory evoked potentials elicited by stimulation of the facial branch of the phrenic nerve. The mapped cortical areas were then stimulated to determine the influence of stimulation of the cortical phrenic projection areas on the inspiratory activity. Above a critical rate, inspiratory bursts were suppressed. Stimulation of the cortical projection areas on the contralateral side were used to determine the influence of the inspiratory discharge on the evoked potentials. The cortical evoked potentials were recorded on an analog tape and also in the 30 cortex of the ansate gyrus.

The inspiratory responses to cortical stimulation were characterized by an inhibition of the firing activity when the stimulation occurred during the first half of the inspiratory burst. The inhibition of the second inspiratory burst was sufficient to momentarily, completely inhibit the inspiratory motor activity. When inspiration was activated late in the second half of the inspiratory burst, off switching of the inspiratory activity was frequently observed to be elicited by the stimulus. Inhibition of the inspiratory activity associated with an increase in expiratory latency. However, at the cortical sites, where the phrenic evoked primary cortical evoked potential was the largest, inspiratory activity was increased by 150% more inhibition of the inspiratory discharge than was elicited by stimulation at other cortical sites. Low threshold sites were located in the postcentral cortex at the intersensory area 3a and 4-gamma and also in the 30 cortex of the ansate gyrus.


Brain and lungs are among the sensitive targets to the toxic effects of hyperbaric oxygenation. The respiratory system in conscious mammals is believed to be primarily affected by oxygen at pressures not exceeding 5 atmospheres absolute (ATA), while at higher pressures, the CNS oxygen toxicity precedes the respiratory effects of oxygen. In previous studies, a close correlation between changes in the regional cerebral metabolic rate for glucose (rCMRgl) and the neurological manifestations of oxygen toxicity was demonstrated in rats exposed to 3 and 5 ATA oxygen (Torbati D., et al, Brain Res., 562:267, 1990; Torbati D. and Lambergen C.J., Brain Res., 241:186, 1981). A significant transient decrease in frequency of breathing (f) is an early characteristic sign of respiratory oxygen toxicity at pressures lower than 2 ATA. However, since the chronic normobaric hyperoxia was tested during development of hyperoxic-induced respiratory changes in neonatal pigs (SPON: S.L. Liles), Inst. For Environ. Med. Univ. of NY 11203 and Schneider Child. Hosp. LIJ-HMC, New Hyde Park, NY. 11040.

We have already shown that pulmonary afferent inputs can have masked effects on PRR activity in neonatal swine (Gootman et al., Ped. Res. 46:912, 1995). In the present report we document the effects of bilateral vagotomy on the high frequency oscillation (of) of phrenic (PRH) and whether afferent vagal fibers alter the response of the respiratory rhythm generator (RRG) to hypoxia. Experiments were performed on 25 pigs between 24 hr and 94 days of age, with a mean weight of 12-16 kg and age (8-15 weeks old at the time of surgery, 2-4 kg/kg/hr during data collection), paralyzed with 5-10 and artificially ventilated on 100% O2 except during hypoxia. Blood gases and temperature were monitored. Monophasic recordings of left and right PRH activity (bandpass 10 Hz to 10kHz) were stored on analog tape along with integrated PRH activity (100 msec time constant), intrathoracic pressure, arterial pressure, EEG and markers indicating phases of artificial ventilatory cycle. Two degrees of hypoxia: 2% and 10% inspired O2 (PaO2 45-65 and 20-30 torr, respectively) were induced by ventilating the animals with gas mixtures containing O2 and N2. The durations of the inspiratory (I) and expiratory (E) phases were calculated from the integrated PRR power spectral density (PSD) sensors were triggered during the I phase on an IBM AT computer. Power spectral densities were measured during the I and E phases of the respiratory cycle. The power spectral density determined the low pass filter setting of 1 kHz. Average power was computed from 200-300 epochs. Framing windows were aligned to reduce stimulus artifacts. The changes in duration of the I and E phases were as expected as those reported in neonatal swine. The duration of the I phase did not change significantly while the duration of the E phase either increased or remained the same. Thus overall respiratory activity was increased. The power spectral energy increased following each inspiration and expiration. The duration of the I phase increased significantly during hypoxia. Bilateral vagotomy did not alter the RRG responses to hypoxia. The overall respiratory activity was decreased as compared to hypoxia.


Three chemosensitive areas on the ventromedial wall of the lateral ventricle (VMS) are involved in the central chemical regulation of respiration. These areas include the rostral (Area-M), intermediate (Area-S), and caudal (Area-L) chemosensitive areas. The rostral area (Area-M) has been implicated in the control of the inspiratory phase, while the intermediate area (Area-S) is involved in the control of the expiratory phase.

In a recent study, we investigated the age-related differences in the activity of chemosensitive neurons in the brainstem of neonatal piglets. We used microelectrode recordings to monitor the neuronal activity in these areas. The results showed that the activity of chemosensitive neurons was highly variable between different piglets and that the activity was influenced by the age of the piglets.

In the newborn piglet, the activity of chemosensitive neurons was low, and the neurons showed little or no response to changes in arterial oxygen and carbon dioxide levels. In contrast, in the older piglets, the activity of chemosensitive neurons increased significantly, and the neurons showed a more robust response to changes in gas concentrations.

The differences in neuronal activity were associated with changes in the expression of chemosensitive receptor genes. In the newborn piglet, the expression of these genes was low, and the neurons showed little or no response to changes in gas concentrations. In contrast, in the older piglets, the expression of these genes increased significantly, and the neurons showed a more robust response to changes in gas concentrations.

These findings suggest that the age-related differences in neuronal activity are due to changes in the expression of chemosensitive receptor genes. The changes in gene expression may be caused by changes in the levels of endogenous opioids, which are known to modulate the activity of chemosensitive neurons. Further studies are needed to determine the exact mechanisms underlying these changes.
457.1 SYNERGISTIC EFFECTS OF DOPAMINE D1 AND D2 AGONISTS ON ROTATION IN STRIATAL SLICES: A MODEL FOR DA AUTORECEPTORS, E.A. Ronay*, E.S. Bahah, D.Clark*, and M.P. Gallmey. (SKF 38393 and apomorphine, 10 μM), which alone produce little or no rotation. Our results suggest that careful titration of DA agonists by selective agonists may be useful in treating Parkinson's disease. We thank the Parkinson Society of Halifax, the Parkinson Foundation of Canada and the Medical Research Council of Canada for support.


To investigate a role for protein phosphorylation as a mechanism for modulation of tyrosine hydroxylase (TH) activity in vivo, the level of TH activity in different striatal regions was investigated. Two dimensional slices (30 mm x 30 mm) were prepared and maintained in a physiological buffer for 1 hour. After preincubation for 30 min with 100 μM nM, the level of TH activity was determined by immunoprecipitation of TH and autoradiography. TH activity was found to be higher in the striatum than in the optic tectum. TH activity was also higher in the substantia nigra than in the striatum. The level of TH activity was also higher in the substantia nigra than in the striatum. These results suggest that careful titration of DA agonists by selective agonists may be useful in treating Parkinson's disease. We thank the Parkinson Society of Halifax, the Parkinson Foundation of Canada and the Medical Research Council of Canada for support.

457.3 SYNERGISTIC EFFECTS OF DOPAMINE D1 AND D2 AGONISTS ON ROTATION IN RATS LESIONED UNILATERALLY WITH 6-HYDROXYPYRAMINE: ANATOMICAL SITES OF ACTION. R.A. Robertson and R.A. Robertson. Department of Pharmacology, Faculty of Medicine, Dalhousie University, Halifax, N.S., Canada B3H 4B7.

At the molecular level, it appears that D1 and D2 receptor agonists have opposite effects. For example, D1 agonists increase release and D2 agonists decrease release of cyclic AMP from striatal slices (Isse, J.-C., and Kebabian, J.-J. Nature 294: 366, 1981). However, D1 and D2 agonists have synergistic effects on turnover when tested on rats unilaterally lesioned with 6-hydroxydopamine (6-OHDA). The level of TH in the substantia nigra is decreased by approximately 90% in rats with 6-OHDA lesions. The level of TH in the substantia nigra is decreased by approximately 90% in rats with 6-OHDA lesions. This decrease is not reversed by DA agonists. However, when D1 and D2 agonists are coadministered, the synergistic effect of DA agonists on TH is attenuated. These results suggest that D1 and D2 agonists have opposite effects on TH activity in the substantia nigra. We thank the Medical Research Council of Canada and the Parkinson Foundation of Canada for support.

457.4 L-DOPA INCREASES DOPAMINE SYNTHESIS IN THE 6-HYDROXYPYRIMINE-LESIONED SUBSTANTIA NIGRA: CORRELATION WITH RotATIONAL BEHAVIOR. T. A. Robertson and R. Robertson. Department of Pharmacology, Faculty of Medicine, Dalhousie University, Halifax, N.S., Canada B3H 4B7.

In order to examine the molecular mechanisms of dopamine (DA) autoreceptors, we have further developed the brain slice model of Wolf et al. (1986) to measure autoreceptor (AR) modulation of tyrosine hydroxylase (TH) activity in slices. Histidase activity, synthesis of DA, and TH activity have been compared in vivo utilizing the HPLC model of Walbers and Roth (1976). In light of this, we have compared our results obtained in vitro to a variety of pharmacological desorbers obtained in vivo.

In the intact animal, the sensitivity of DA synthesis to exogenous DA (after inhibition of decarboxylase with MBD-1015) is linear with protein and tissue, temperature dependent, and increased 90-100% in the presence of 30 mM K+. After inhibition of dopamine synthesis for 30 min, 90% of total intracellular, 90% of total dopamine extracellular, and DA is evenly distributed between the two compartments. Thirty mM K+ induces DA release and increases the extracellular concentration of DA from approximately 30 nM to 300 nM. Under basal conditions, DA is released from the slice and DA uptake is a function of the size of the compartment. This size is affected by the concentration of DA in the slice and by the concentration of DA in the extracellular fluid. These circumstances, agonists such as quinpirole and pergolide fully reversed DA uptake to levels below K+ control. Furthermore, the effect of SKF 38393 was attenuated in the presence of nonselective, or 230nM. The inhibition of DA synthesis by quinpirole (3 μM) was potentiated in the presence of SKF 38393, suggesting separate sites of action for these two compounds. Together the data suggest that 1) autoreceptor regulation of DA synthesis is parallel with the in vivo uptake of DA and 2) issues of intrinsic agonist efficacy, D1-D2 receptor coupling, and receptor-effector coupling can be investigated in vivo by pharmacological means. Supported by NIMH 41227, NIDA 04120, UPF, MPAA, and the State of Michigan-DMH.

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457.5 STUDIES ON THE IN VIVO CATABOLISM OF EXOGENOUS DA AS INFUSED THROUGH A PUSH-PULL CANNULA IMPLANTED IN THE RAT CAUDATE NUCLEUS. G. D. Chang and J. L. Ramirez. Department of Physiology and Biophysics, University of Pittsburgh, Pittsburgh, PA 15260.

Determination of the levels of dopamine (DA) metabolism in the brain has provided pivotal information regarding how central acting agents alter Dcnergic activities and DA metabolism. Recently two important techniques have been widely used to measure in vivo efflux of DA from discrete areas: intracerebral dialysis and push-pull perfusion techniques. Coupled to HPLC with electrochemical detection, these techniques allow measurement of the rate of monoamine metabolism simultaneously and more importantly allow continuous sampling in freely behaving animals. In the present study, we have adopted the push-pull perfusion and HPLC-EC technique to study the effects of exogenous DA on the output of neuromodulators released from the caudate nucleus (CN) of freely behaving rats.

Push-pull cannula of 22 gauge were aimed at the CN of adult male rats according to the deGroot's atlas. Ten to twenty days after implantations, modified Kreb-Ringer phosphate medium (KRP), or 1.4%, was infused into the CN cannula at a flow rate of about 12 μl/min. Puffs of 15 min intervals were subjected to HPLC-EC analysis of DA, 3,4-dihydroxyphenylalanine (DAOPA), 5-hydroxytryptophan (5-HTP), and 5-hydroxyindoleacetic acid (HVA). Six animals received local infusions of 10-6 M DA in KRP for 15 min at 1400h and 1600h, respectively. Another four rats received two infusions of 5×10-5 M DA in KRP at 1400h and 1600h, each for 15 min. However, 30 min after the first infusion of 5×10-5 M DA, 10-3 M Nomifensine was included in the KRP until the end of perfusion.

Infiltration of exogenous DA at these three concentrations elicited significant increases in DAOPA output from all animals. The total amounts of DAOPA increases induced by 10-6 M, 5×10-5 M and 10-5 M DA were 1218.6, 267.4 ± 213, and 267.4 ± 685 pg, respectively. In addition, the higher two doses of exogenous DA also induced increases in HVA output. The increases in DAOPA output of 5×10-5 M DA was reduced by 10-3 M Nomifensine in KRP. Interestingly exogenous DA-induced increases in DA output were little affected by the Nomifensine treatment. Furthermore, infusions of exogenous DA did not change 5-HTP output. In conclusion, our results confirm in vivo that a DA catabolic pathway via the aromatic interaction decreases in the extracellular space which, despite the apparent inhibition of DA uptake, does not appear to affect serotonergic activities.

457.6 L-DOPA-INDUCED INCREASE IN DA RELEASE FROM STRIATUM: IN VIVO STUDIES ON MECHANISM OF ACTION. G. L. Snyder and M. J. Zigmond. Department of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

L-Dihydroxyphenylalanine acid (DOPA) is effective in reversing the neurochemical deficits caused by nigrostriatal bundle degeneration, apparently by increasing the availability of dopamine (DA) in brain. The current experiments examine the location at which DOPA-induced increases in DA efflux (from 10-6 M DA) or tyrosine (10-5 M) are added to the superfusion buffer and 60 min later slices are exposed to electrical field depolarization (100 μA, biphasic pulses) delivered over the next 15 min for 15 minutes. Superfused and tissue slices were analyzed for endogenous DA by electrochemical detection. Data were expressed as DA overflow (stimulated minus basal DA efflux) in pg per mg protein.

Superfusion with DOPA showed a significant elevation in the DA content of striatal slices (basal DA content: 68.6 ± 6.4%) increase due to DOPA: (21.6 ± 6.4%) as well as a large increase in DA release (126.9 ± 26.9%) with a corresponding stimulation of 93% of the dopaminergic terminals in striatum (basal DA content: 4.9 ± 0.9 pg/mg; increase due to DOPA: +14.2 ± 0.9 pg/mg). However, 6-DA reduced by 83% the increase in stimulation-evoked overflow of DA normally produced by DOPA. In order to determine if the increase in DA overflow in the striatum was Ca2+-dependent, intact striatal slices were stimulated during superfusion with a Ca2+-free containing Krebs medium (0.1 mM), while the stimulation-evoked overflow of DA from control slices was abolished in Ca2+-free medium (-97%), the increase in DA content in Ca2+-free medium was reduced (-36%). These data are consistent with our previous findings on the in vivo and in vitro release of DA during exposure to DOPA (Neller et al., Soc. Neurosci. Abst. 1986). Furthermore, they suggest that the conversion of DOPA to dopamine in DA occurs preferentially in the site that release occurs by both Ca2+-dependent and Ca2+-independent mechanisms. (Supported in part by USPHS grant MH30960.)


The neurochemistry and physiology of the nigrostriatal (DA) and mesoaccumbens (DA) systems in the CNS is increasingly recognized to represent the expression of the function and integrity of DA systems of the nigrostriatal and mesoaccumbens populations. Consistent with this organization, optimal pharmacotherapy would reflect DA neuronal population-selective drug actions. However, our preliminary in vivo study and study of DA neuronal systems have been hindered by the confounding, uncontrolled influences characteristic of in vivo studies and the uncertain relevance of in vitro studies. As a crucial methodological compromise, we attempted to isolate and study two distinct DA neuronal populations in the anesthetized and functionally continuous cell body-to-territorial relationship.

Male Sprague-Dawley rats were decapitated, their brains were removed and two coronal cuts were made, the first at the level of the rostral head on the caudate nucleus, the second just anterior to the pons, a sharpened stainless-steel cannula (0.6 mm, i.d.) attached to a mechanical vibrator was guided through the tissue attachment to a predetermined route to collect cell bodies at the lateral edge of the ventral tegmentum (A-10, for the mesoaccumbens projection) or the substantia nigra pars compacta (A-9, for the nigrostriatal projection), their anesthesia running through the dorsal aspect of the midline into the terminal cannulae or caudate putamen, respectively. The cylindrical expansions were the bottoms of a push-pull chamber. The rostral 3 mm was inserted into a 1.0 mm diameter plastic collection tube with which the incubation medium bathing the nerve terminals was drawn off via a peristaltic pump. Samples were collected over an 8 minute period and then analyzed by HPLC-EC for dopamine release. During the last 2 min of the 8 min collection period, dopamine efflux which was augmented by co-application of the dopamine receptor blocker, haloperidol (10 nM). These effects were blocked by ephedrine perfusate, indicating the involvement of mono- and -dendritic as opposed to terminally-localized neurotransmitter receptors. Thus, we have demonstrated viability, functional coupling and specificity of these systems and are continuing to investigate the neurotransmitter results of other putative peptide and non-peptide neurotransmitters on this model.


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457.11 ACUTE EFFECTS OF AMPHETAMINE AND COCAINE ON CATECOLAMINE RELEASE FROM STRIATAL AND HYPOTHALAMIC REGIONS. B. A. Bennett, N. Morris*, D. K. Sundberg*, Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27103.

The tuberoinfundibular neurons of the hypothalamus differ from those found in the striatum in that they have much shorter axons and do not appear to possess either presynaptic (D-2) autoreceptors or a high affinity amine uptake system. In the following experiments two antagonist drugs were used to induce release or inhibit uptake of the catecholamines, norepinephrine (NE) and dopamine (DA). This study was performed to demonstrate in vitro the differences between catecholamine regulation in these two independent dopaminergic areas.

Striatal and hypothalamic areas were obtained from Sprague Dawley male rats (150-300g). Tissues were pre-incubated in Earle's, pH 7.4 in 3% O2/5% CO2 at 37°C for one hour. Four subsequent one hour incubation periods were conducted from the following protocols: control (drugs at 10^-5 M), control, and followed by a 50 mM potassium. Media and tissue samples were acidified and catecholamines were alumina extracted. Catecholamine concentrations were obtained using HPLC with electrochemical detection. Shown in the table are the levels of DA and NE expressed as percent increase over control periods (n=4, *not significantly different from control).

<table>
<thead>
<tr>
<th>Compound</th>
<th>AMPH</th>
<th>COC</th>
<th>CKB</th>
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<tbody>
<tr>
<td>Hyp.</td>
<td>360</td>
<td>120</td>
<td>200</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>320</td>
<td>210</td>
<td>750</td>
</tr>
<tr>
<td>Str.</td>
<td>200</td>
<td>270</td>
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<tr>
<td></td>
<td>160</td>
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<td>110</td>
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Amphetamine causes the release of NE and DA in both areas. On the other hand, cocaine augments media levels of dopamine in the striatum only whereas NE levels are increased in both areas. This data would indicate that cocaine inhibits NE uptake in both areas whereas dopamine is inhibited in the striatum and not in the hypothalamus, as would be expected if there were no high affinity amine uptake pumps present. The potassium period was included to show the magnitude of catecholamine release resulting from nerve depolarization and suggests that multimodal interactions are occurring particularly in regards to NE release in the hypothalamus. This work was supported by NIH grants NS-22492 and NS-24723.

Department of Neurobiology and Anatomy, Univ. of Texas Med. Sch., Houston, TX 77225 and Dept. of Pharmacology, Harvard Med. Sch., Boston, MA 02115.

Immunocytochemical studies have demonstrated the presence of a vasointestinal peptide (VIP)-like immunoreactivity in bovine adrenal medulla in newborn calves. Although the site of action and the mechanism of this immunoreactive material is not known, the finding that VIP increases DOPA synthesis and DOPla counts in cultured bovine adrenal medullary cells (Tischler \textit{et al.}, Neurosci. Lett. 61:141, 1985) suggests that it may play a role in the regulation of catecholamine biosynthesis. In the present study, we have examined the effects of VIP on catecholamine synthesis in suspensions of bovine adrenal chromaffin cells and characterized the VIP-induced activation and phosphorylation of tyrosine hydroxylase (TH).

Catecholamine synthesis rates in intact chromaffin cells were estimated by \textit{CO}2 evolution following the decarboxylation of DOPA formed from \textit{C}1-C tyrosine. Addition of VIP to the cells accelerated synthesis in a dose-dependent manner. Maximal stimulation was 3-4 fold over basal levels and was achieved at 10 \textit{nM} VIP. The actions of VIP were relatively specific since other peptides related to VIP (secretin, glucagon and PHI) had no effect on synthesis. To measure TH activation, cells were sonicated and gel filtered aliquots assayed for enzyme activity at \textit{pH} 7.0 in the presence of substrates cofactor (0.10 \textit{mM} 6-\textit{Mg}). The results showed that TH activity was elevated 3-fold after treatment of the cells with 10 \textit{nM} VIP. This activation was dose-dependent and occurred over the same concentration range as that observed for the effects of VIP on synthesis in intact cells. Enzyme activity reached maximal levels one minute after one minute of treatment and had declined only slightly in cells exposed to VIP for up to 15 minutes. In intact cells pretreated with 10 \textit{nM} VIP, the enzyme showed a 3-fold increase in the incorporation of 32P into the 60 K subunit of TH. HP-EC analysis of the tryptic fragments of TH revealed an increase in the 32P content of a single phosphoprotein (Sol. Neurosci. Abstr., 1987). Since this is the same peptide phosphorylated by CPI and forskolin, additional experiments were carried out to determine whether VIP affected 


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Considerable evidence supports the concept that in situ activation of tyrosine hydroxylase (TH) is associated with phosphorylation of one or more sites of the enzyme. In this report, we use the site of the enzyme to characterize the number of sites on TH phosphorylated in situ in the intact cell and map the relationship between the sites phosphorylated and the activation of TH in response to acetylcholine (ACH).

Acetylcholine greatly increased the amount of tyrosine hydroxylase phosphorylated in situ by incubation of the cells with 10 \text{nM} for 90 min, revealed 7 phosphopeptides when subjected to HP-EC using a 0.175 acetic acid gradient in 0.13 TIA (peptides were numbered 1 through 7 according to their order of elution). An identical chromatographic profile was obtained for tryptic fragments of immunoprecipitated 32P-TH, indicating that all 7 phosphopeptides of the 60 K band were derived from TH. Because rechromatography of each peptide in a second HP-EC solvent system yielded single radioactive peaks, each peak appeared to contain only one labeled peptide. Cells were incubated with 100 \text{nM} ACH, the phosphate content of 5 phosphopeptides (P3-7) was increased. Time course studies 2 phosphorylation patterns in response to ACH. A rapid 5-fold increase in the 32P content of peptides 3, 5 and 6 occurred which became maximal in 10-15 min and returned to basal conditions by 2-3 min, present time is still present in the incubation medium. A slower increased 3-fold incorporation occurred in peptides 4 and 7 which reached a plateau in 4-5 min and gradually returned to basal conditions by 16 min. The addition of ECTA 10 min prior to ACH blocked the enhanced phosphorylation of all 5 peptides, indicating that the in situ phosphorylation of TH by ACH is calcium-dependent. To determine the relationship between the multiple site phosphorylation of TH and enzyme activation, cells were treated with 100 \text{nM} ACH and TH activity measured in vitro after sonication of the cells. The enzyme was maximally activated by 15-30 sec, and the addition of ACh caused enzyme activity to decline rapidly, approaching basal levels by 4 min. A comparison of the time course for TH activation vs phosphorylation indicated that activation of TH was paralleled by a greater incorporation into peptides 3, 5, and 6, whereas there was no inverse relationship between TH activation and enhanced phosphorylation of peptides 4 and 7. Supported by USPH NS 11061.


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It has long been known that changes occur in concentrations of neurotransmitters in the developing nervous system (CNS). Growth of biogenic amine-containing neuronal systems within the spinal cord is an important developmental event. Therefore, the presence of biogenic amines and their metabolites at different stages of development could aid in the determination of the role played by these chemicals in the development of the CNS. We have previously reported a large decrease in 5HT and NE levels in the hindlimb regions of the spinal cord at the last two gestational stages (Purpura et al., Brain Res. 127:205, 1977). In the present study, we have investigated the levels of 5HT, DA and NE in the developing guinea pig. In the fetus, 5HT and NE levels were high, while DA levels were low. However, at birth, the levels of 5HT and NE were decreased, while NE levels were increased. This pattern was observed at all levels of the spinal cord, including the lower and upper levels of the cord. The results suggest that the development of the spinal cord follows a specific pattern that is different from that observed in the peripheral nervous system.
458.3 STIMULATION OF DORSAL RAPHE NUCLEUS (DRN) AND LOCUS COERULEUS (LC) INCREASES 5-HTP ACCUMULATION IN MEDIAL PREFRONTAL NUCLEUS (mPFC) BUT NOT IN MEDIAN Eminence (mE).
L. Paternak*, R. D. Hartman* and C. A. Barracough (SPON: L. Joh and H.W.M. Steenbush, Neuro-Behavioral Research Unit and Department of Anatomy, University of Helsinki, Finland, Department of Neurology, Cornell University Medical College, New York, USA, 4Department of Pharmacology, Free University, Amsterdam, The Netherlands.
In our previous study (J. Auton. Nerv. Syst., 15:21, 1986) it was shown that a population of principal neurons in LC containing 5-HT and tyrosine hydroxylase immunoreactivity (5-HT/Tyr-OX) exhibit 5-hydroxytryptamine immunoreactivity during early postnatal development. In this study, indirect immunofluorescence was used to examine the immunohistochemical expression of serotonin (5-HT) in principal sympathetic neurons in the superior cervical ganglion (SCG) of normal and thyroid-deficient (hypothyroid) rats. The experiments were performed on postnatal day 4 (PD4) and PD7, 10, 14, 21, and 28, and adult animals. The following results were observed: (1) the number of 5-HT/Tyr-OX neurons was significantly lower in the hypothyroid group compared to the normal group; (2) the number of 5-HT/Tyr-OX neurons was significantly lower in the hypothyroid group compared to the normal group; (3) the number of 5-HT/Tyr-OX neurons was significantly lower in the hypothyroid group compared to the normal group; and (4) the number of 5-HT/Tyr-OX neurons was significantly lower in the hypothyroid group compared to the normal group.

458.4 IMMUNOCYTOCHEMICAL LOCALIZATION OF 5-HYDROXYTRYPTAMINE AND TYROSINE HYDROXYLASE IN SYMPATHETIC NEURONS.
J. Happula, T. Soinila, T. Tahtinen*, J. T. J. Joh and H.W.M. Steenbush, Neuro-Behavioral Research Unit and Department of Anatomy, University of Helsinki, Finland, Department of Neurology, Cornell University Medical College, New York, USA, 4Department of Pharmacology, Free University, Amsterdam, The Netherlands.

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We have begun a series of studies to examine whether ES of DRN or LC affects 5-HT secretion in hypothalamic regions which contain LHRH cell bodies (mPFC) and terminals (mE). To evaluate changes in 5-HT secretion we measured the accumulation of 5-hydroxytryptophan (5-HTP) following blockade of L-tryptophan decarboxylase with NBD-1015. Levels of 5-HTP were measured in various regions of the brain, including the mPFC and mE, at 0, 10, 20, 30 and 40 min after NBD treatment. A linear accumulation of 5-HTP occurred over 30 min and 5-HIAA, DA, E and DOPAC decreased during the first 30 min after NBD treatment (STIM-DRN). None of the control rats, but surprisingly, no differences occurred in mE 5-HTP accumulation in a manner similar to that observed after 5-HT treatment but, again, no changes occurred in mE 5-HTP levels.

These data suggest that: (1) 5-HTP accumulation from DRN project to mPFC but not to mE; (2) LC-ES activates both NE and 5-HT neurons whose axons reach LHRH containing regions in mPFC. Thus, an increase in activity in one system (e.g. LC) could release both NE and 5-HT in mPFC. Now LHRH neurons respond to the dual signal input received to be released. Supported by Research Grand HD-02138.


Sympathetic ganglia are composed of principal nerve (PN) cells and small intensely fluorescent (SIF) cells. Evidence has been provided that SIF cells may function as interneurons, endocrine cells or chemo-receptor cells. In addition to catecholamines, 5-hydroxytryptophan (5-HT) has been detected in the rat superior cervical ganglion. During early postnatal development, 5-HT immunoreactivity is observed both in PN cells and SIF cells. However, if adult rats are treated with nialamide and loaded with 5-HT-immunoreactive SIF cells were still seen in ganglia of fluoxetine-treated rats indicating that the 5-HT is likely to be synthesized in the SIF cells. Thus, the results are in some conflict with the origin of 5-HT in sympathetic cells. To study directed to determine the mechanism of origin of 5-HT in sympathetic cells.


The primate neocortex receives a rich serotoninergic input from neurons located in the raphe complex. In the present study, immunocytochemical methods and an antibody directed against serotonin were used to map the laminar distribution of serotoninergic fibers in the anterior and posterior segments of the cingulate cortex (Brodmann areas 23 and 24) of the rhesus monkey (Macaca mulatta). Cortical laminae were first identified in Wister rat preparations: the anterior cingulate cortex is characterized by a thin layer I, the absence of layer-IV neurons, and densely stained, large cells in layers V and VI; and the posterior cingulate cortex has six distinct layers, with a clearly identifiable layer IV. In serotonin immunocytochemical preparations, at least two types of fibers were identified: fine-diameter axons with either small round or smooth, fusiform varicosities; and larger-diameter axons with large spherical varicosities. Both types of axons were seen in both anterior and posterior cingulate cortices. The anterior cingulate cortex shows a denseplexus of fibers in layers I and II; the majority of fibers in layer I course parallel to the pial surface, whereas those in layer II are more radial towards the pial surface. Serotonergic fibers are more sparse in layers III-VI. Serotoninergic processes appear to enter anterior cingulate through the cingulum bundle, pass through layers V and VI, and terminate principally in superficial layers. In contrast, serotonergic fibers in the posterior cingulate cortex through layer V, are densely packed and radially oriented towards the pial surface in layers III-V, and are more sparse in the central laminae of the posterior cingulate cortex. The results of this study suggest that serotoninergic neurons of the raphe projecting to the anterior cingulate cortex exhibit distinctively different patterns. The anterior and posterior cingulate cortices may, therefore, be further differentiated by transmission electron microscopic, as well as by morphological and connectivity criteria.
458.7 IMMUNOHISTOCHEMICAL LOCALIZATION OF 5-HYDROXYTRYPTAMINE IN SMALL INTENSELY FLUORESCENT CELLS IN PROG SYMPATHETIC GANGLIA. Karin Vane Jorgensen and Roy B. Denney. Department of Anatomy, Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada.

5-Hydroxytryptamine (5-HT) has been shown to play important modulatory roles in the amphibian sympathetic ganglia, including regulation of acetylcholine (ACh) release, control of ACh receptor sensitivity, augmentation of slow synaptic responses, as well as having direct hyperpolarizing action on neurones. However, it is not known if these modulatory effects are present in vivo. One way that 5-HT may affect the ganglia is via circulation. Alternatively, 5-HT may be localized in specific structures in the ganglia and may act locally. To our knowledge no report to date has described the localization of 5-HT in amphibian sympathetic ganglia. We have used immunohistochemical techniques to study localization of 5-HT in the preganglionic nerve trunks and 9 and 10 sympathetic ganglia of the bullfrog Rana pipiens. The tissus were immersion-fixed in 4% paraformaldehyde, cryostat and paraffin sections were cut and processed using both Coons’ indirect immunofluorescence method and Sternberger’s peroxidase-antiperoxidase method. We detected no immunohistochemical reactivity for 5-HT in either preganglionic nerve trunks, or in the principal ganglionic neurons. However, intense 5-HT immunoreactivity was seen in clusters of small intensely fluorescent (SIF) cells in ganglia 9 and 10. The localization of 5-HT to SIF cells in frog sympathetic ganglia opens up the interesting possibility that SIF cells may be involved in the above-mentioned modulation of ganglionic transmission by releasing 5-HT.

458.8 DISTRIBUTION OF MONOAMINERGIC NEURON TERMINALS IN THE OPTIC TECTUM OF GYMNOSOMATID FISH. AN IMMUNOFLOUORESCENCE AND IMMUNOCYTOCHEMICAL STUDY. E. Sas and L. Maler. Dept. of Anatomy, Faculty of Health Sciences, Ottawa, Canada, R1K 8M5.

Our previous studies investigated the diverse cell types of the optic tectum of gymnoid fish, the sources of tectal afferents, their laminar segregation, and the stratified distribution of acetylcholinesterase containing axons in the optic tectum. The present work deals with the identification of monoaminergic terminals in the optic tectum of Apterurus leptocephalus with the aid of: 1) Immunofluorescence technique; 2) Immunocytochemical method for the differentiation of dopamine from noradrenaline containing fibers. The protocol of Smets et al. was followed, using antiserum raised against dopamine and dopamine-beta-hydroxylase kindly provided by Dr. R.M. Buijs’s laboratory. 3) The immunocytochemical method of Halasz et al. 1978, was used for identification of serotoninergic fibers.

In the optic tectum of Apterurus, serotonin containing axons were present in the superficial sublamina of stratum opticum, at the stratum opticum/stratum fibrosum et griseum superficiale border, and dorsal part of mid stratum griseum centrale. In addition, some thicker fibers were observed ascending through stratum album centrale, whereas in the stratum periventriculare they formed small bundles which were obliquely cut, in transverse sections. Among the possible candidates for sources of serotoninergic terminals in the tectum are the N. raphe dorsalis, and the mesencephalic dorsal tegmental nucleus. This model project to the tectum and part of their neuronal population showed strong immunoreactivity for serotonin.

Dopaminergic fibers were distributed as follows: a) In a bilayered fashion in stratum opticum, b) As a loose plexus at the deep stratum fibrosum et griseum superficiale border, c) as ascending fibers in stratum griseum centrale, and d) forming a meshwork in stratum periventriculare. The dopaminergic fibers appeared to be confined mainly to the known retinorecipient layers. Although there is no report in the literature of the existence of dopaminergic fibers in fish. Recent studies (Bökfelt et al 1984) have shown a subpopulation of dopamine containing ganglion cells in the retina, thus supporting our findings of possible dopaminergic retinotectal projections in Apterurus.

In order to test the hypothesis that the distribution of dopamine-like immunoreactive fibers is an accurate reflection of the distribution of monoaminergic neurons in the optic tectum of Apterurus, we compared with reports in other fish and the possible relevance of these neurotransmitters in the modulation of sensory–motor processing will be discussed.

458.9 [3H]TRYPTAMINE BINDING SITES IN BRAIN: LACK OF ASSOCIATION WITH MONOAMINE OXIDASE ENZYMES. A.M. Martino-Barrows, D.C. Perry, L. Grimes, M.L. Jones, and K.J. Kellar. Departments of Pharmacology, Georgetown University School of Medicine and George Washington University School of Medicine, Washington, DC.

[3H]Tryptamine labels high affinity binding sites in rat brain with a unique pharmacological profile (Casco and Keller, Eur. J. Pharmacol.: 95: 31, 1983). In vitro autoradiographic techniques demonstrated a distinct heterogeneous distribution in rat brain, and it also revealed the presence of a second class of tryptamine sites (S-2') in choroid plexus (Perry, J. Pharmacol. Exp. Ther.: 236: 546, 1986). It remains unclear, however, whether [3H]tryptamine is binding to a true receptor or perhaps to membrane-bound monoamine oxidase (MAO) enzyme. In homogenate binding experiments, it was found that the presence of paraglyline (a MAO inhibitor) is required for specific binding. Optimal binding occurs with 30 µM paraglyline; higher concentrations decrease binding, with an IC50 of approximately 400 µM. This suggests that lower concentrations of paraglyline protect the radioligand from enzymatic destruction, while at higher concentrations it does paraglyline begin to compete at the tryptamine binding site. Comparisons with paraglyline, however, are complicated by the non-competitive nature of its interaction with MAO. A better comparison is with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), which like tryptamine is an MAO substrate. As shown in binding experiments, with an IC50 of approximately 20 nM, the IC50 of MPTP vs. [3H]tryptamine binding at 2-3 nM potency, whereas the IC50 of tryptamine vs. [3H]MPTP is >1000 nM. Similarly, while [3H]MPTP has a Kd of approximately 20 nM, the IC50 of MPTP vs. [3H]tryptamine is >1000 nM. Finally, the autoradiographic distribution of [3H]tryptamine, [3H]paraglyline and [3H]paraglyline in rat brain were compared. Although similarities were seen, a number of significant differences were readily apparent. In addition, we were unable to rule out the possibility that a component of [3H]tryptamine binding is to membrane-bound MAO, the present data argue strongly for an independent identity for tryptamine binding sites.

458.10 CELLULAR DISTRIBUTION OF MONOAMINE OXIDASE A IN INTESTINAL EPITHELIUM IS CONSISTENT WITH ITS HYPOTHESIZED PROTECTIVE ROLE AGAINST VASODUCTIVE AMINES. Ellen L., L. Parker, E. Vargas, A. A. M. Vaghaspshak and Denney, R. M. Department of Human Biological Chemistry and Genetics, Graduate School of Biomedical Sciences, University of Texas Medical Branch, Galveston, TX.

Patients treated with inhibitors of monoamine oxidase (MAO) A sometimes experience adverse experiences during the first dose. It is thought that these reactions are caused by the inhibition of monoamine oxidase A (MAO-A) and the consequent inhibition of the catabolism of amine compounds, such as amphetamine and tyramine. One of the proposed mechanisms by which these reactions are induced is the liberation of free tyramine from storage sites by ingestion of foods containing dietary tyramine (the so-called "cheese effect"). For this reason, it has been proposed that intestinal MAO A provides an effective metabolic barrier to vasoactive amines in the diet. To investigate this hypothesis, we have localized MAO A in histological sections of human ileum using a newly-identified monoclonal antibody to human placental MAO A (R&D Systems), which in contrast to previously-reported monoclonal antibodies to human MAO A (Kochsperger et al. J. Neurosci.: 5:2874, 1985). Like the previously-described antibodies, MAO A-4D3 immunoprecipitates Trlton X-100-solubilized MAO A with little inhibition of catalytic activity. However, at equivalent dilutions of antisera, MAO A-4D3 stains MAO A in immunoblots of the purified enzyme or Triton X-100 extracts of placental mitochondria more intensely than any of the other antibodies. Competitive binding experiments indicate that MAO A-4D3 and two previously isolated MAO A-specific antibodies (MAO A-TH10 and MAO A-TH3) from different BALB/c mice recognize overlapping or identical binding sites on MAO A. Immunohistochemical analysis of histological sections of normal human ileal tissue reveals marked staining only in the epithelial layer of the villi, consistent with the proposed physiological role of MAO A as a metabolic barrier in the intestine. In contrast, immunocytochemical analysis of histological sections of the ileum from a patient with Marfan syndrome, who carries a known mutation in the functional MAO A gene, revealed little staining. The presence of MAO A in enterocytes, which constitutes a single-cell layer separating the lumen from the outside of the body, is strikingly reminiscent of the localization of MAO A in the synaptotrophoblast of placental villi (Thorpe et al. J. Histoch. Cytochem.: 35: 123, 1987) at the boundary between mother and fetus. These results suggest that additional epithelial cell and tissues should be examined to determine if MAO A expression is a common feature of epithelial "barrier" tissues. [Supported by NIH 59543]
458.11 PHOTOSTRAINACTIVATION OF MONOMINE OXIDASE A (MAO-A) AND B IN HUMAN BRAIN. M. C. Snow, P. K. J. Schenck, and H. L. Whish Division of Biological Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA 90033

The effects of 4-fluoro-3-nitrophenyl azide (FNP) on monoamine oxidase (MAO) A and B in human brain cortex were studied. In the dark, FNP competitively inhibited both MAO-A (K_i=4.5 μM) and MAO-B (K_i=2.0 μM) with similar affinities. Upon irradiation of increasing concentration of FNP with the brain homogenate, the V_max was decreased whereas the Km values were not affected. This result suggests that a covalent linkage was formed between the enzyme and the photolyzed FNP, and that the FNP binding site may be the same as the substrate. Additional evidence for the photoinduced covalent binding of FNP to both MAO-A and MAO-B was that there was no recovery of activity upon extensive washing of the photolyzed FNP enzyme complex. Interestingly, the photoprotein inhibitory effect of FNP on MAO-B was much more effective than MAO-A. A 50% photoprotein inhibition of MAO-B activity was obtained with 1-2 μM FNP, whereas 15-30 μM was required for MAO-A. These results suggest that there is a conformational or a structural difference in the active sites of the two types of MAO, and FNP may be useful for probing this region of the active site. (Supported by NIMH Grant No. 19702 and 390855)

458.13 MULTIPLE ADENOSINE RECEPTORS IN THE RAT VAS DEFERENS PREPARATION. Susan J. Ward and Renee Cary, Department of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, New York, 12144.

It is generally recognized that adenosine (A) agonists inhibit the electrochemically stimulated release of norepinephrine in the rat vas deferens preparation. Studies evaluating potential CRH actions of A agonists (e.g., analogues, antagohistines action) prompted the present study which was designed to characterize receptors located on nervous tissue in an isolated preparation. Vas deferens preparations (RVD) were stimulated electically at 0 Hz and bathed in warm oxygenated Krebs solution. A agonists and some antagonists inhibited contractions of the RVD. IC50 values are shown in Table 1. Reported selectivity for A1 and A2 receptors is shown in Table 1. The following compounds were evaluated for their effects on the inhibition of adenosine agonists: the A2 agonist 8-PT, DPX, and PACPX, the phosphodiesterase (PD) inhibitor rolipram, and the mixed PD inhibitor antagonist theophylline.

<table>
<thead>
<tr>
<th>Adenosine</th>
<th>Selectivity</th>
<th>8-PT</th>
<th>DPX</th>
<th>PACPX</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAA</td>
<td>0.19</td>
<td>7.04</td>
<td>7.04</td>
<td></td>
</tr>
<tr>
<td>CPA</td>
<td>0.12</td>
<td>7.11</td>
<td>7.07</td>
<td></td>
</tr>
<tr>
<td>NECA</td>
<td>0.13</td>
<td>7.17</td>
<td>7.59</td>
<td></td>
</tr>
<tr>
<td>(–) PIA</td>
<td>0.28</td>
<td>7.13</td>
<td>7.17</td>
<td></td>
</tr>
<tr>
<td>2-CAOD</td>
<td>0.47</td>
<td>7.18</td>
<td>7.17</td>
<td></td>
</tr>
<tr>
<td>(–) PIA</td>
<td>0.47</td>
<td>7.18</td>
<td>7.59</td>
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</tbody>
</table>

Theophylline, 30 and 100 μM produced a small, non-competitive rightward shift in the concentration-effect curves for each of the agonists, but at 300 μM, either had no effect, or potentiated the agonists. These potentiating actions were mimicked by rolipram (10 μM). Both theophylline and rolipram inhibited twitch height in the RVD, whereas the non-PD inhibitors 8-PT, DPX, and PACPX did not potentiate A agonists or inhibit twitch height. pA2 values for 8-PT = 30-37 μM to antagonize the A1 selective agonist CAA, CPA, (–) PIA, and CHA with the mixed A1/A2 agonist NECA ranged from 6.8 and 7.1 pA2 values for 8-PT to 7.1-7.3 pA2 values for CPA (Table 2). For both antagonists, the slopes of the ordinate plots for the antagonism of the mixed A1/A2 agonist 2-CAOD were less than 1. The A1 selective antagonist PACPX (IC50 = 0.3 μM) antagonized CPA, (–) PIA, and CHA with pA2 values ranging from 7.5 to 8.1. These concentrations of PACPX produced less than 3-fold shifts in the IC50 for 2-CAOD and NECA. Higher concentrations of PACPX did antagonize 2-CAOD and NECA, the pA2 value for 2-CAOD-PACPX being 6.5. The data caution against the use of A antagonists as possible pharmacological tools in these cases. The data also suggest existence of 2 populations of receptors in the RVD that are consistent with an A1/A2 classification.

458.14 SELECTIVE LOCALIZATION OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS IN MOUSE BRAIN. L. Lim, R. Waldner, F. Gillar, and B. Drujan, Department of Medical Microbiology, University of Southern California, Los Angeles, CA 90033

Previous studies have shown that the antidepressant, phenelzine (PLZ) is more potent behaviourally and biochemically when its alkyl side chain is deuterated. The increased behavioral efficacy of the antidepressant properties of 3,4-dichloro-L-phenylalanine (3,4-DCL-PALA) appear to be correlated to increased levels of brain tryptophan (TRP) and p-phenylethylamine (PEA) rather than to other monoamines, such as serotonin (5-HT), dopamine (DA) or noradrenaline (NA). Selective studying the effects of chronic administration of 3,4-DCL-PALA showed that increased DA, NA and 5-HT levels more than control. This is the difference between the drugs and PALA or PALA or PALA or PALA. We have extended this study by measuring the effects of chronic PLZ and [2H2]-PLZ on brain NA, DA, 5-HT, TRA, PEA, m-tyramine (mTA), p-tyramine (pTA) levels and rat brain 5HT activity. Saline, PLZ, or [2H2]-PLZ (0.5 or 2.5 mg/kg) were administered twice daily (s.c.) for 13 days via mini-osmotic pumps. After this, the rats were killed and the striata dissected out. Monoamines, tyrosine, tryptophan, and metabolite levels were measured simultaneously using HPLC-EC and mass spectrometric methods on aliquots of the homogenized striatal samples. Tyrosine and tryptophan levels were unaffected by PLZ or [2H2]-PLZ. The concentrations of DOPAC, HVA and HIAA were dose-dependently decreased by PLZ and [2H2]-PLZ; furthermore, [2H2]-PLZ decreased the amount of these acids more than the equivalent dose of PLZ. NA levels were increased the same amount (130% of control) by all drug treatments; i.e., no dose or deuterium effects were observed. With the exception of PEA, qualitatively similar effects were found with all other amines measured; their amounts were increased dose-dependently and the effects of [2H2]-PLZ were greater than those of PLZ. While the levels of NA, 5-HT and 3-methoxytyramine were increased 6.3, 3.1 and 6.8 times greater than control by 2.5 mg/kg [2H2]-PLZ, the levels of TRA, mTA and pTA were increased even more (13.1, 12.0 and 10.1 times, respectively). In contrast, PE A levels were not increased by PLZ and only 2.5 mg/kg [2H2]-PLZ had increased 2.3 fold. Rat cerebral MAO activity was inhibited dose-dependently by PLZ and [2H2]-PLZ. Type A MAO was inhibited more than type B, and [2H2]-PLZ inhibited both types more than PLZ. The present study agrees with previous findings. In addition, it shows that the efficacy of PLZ was increased about 5 fold by deuteration, that [2H2]-PLZ inhibited MAO more than PLZ, and that [2H2]-PLZ increased TRA, mTA and pTA levels more than other monoamines. PLZ levels were least affected by the drug treatments, and that [2H2]-PLZ increased TRA, mTA and pTA levels more than other monoamines. PLZ levels were least affected by the drug treatments, and that [2H2]-PLZ increased TRA, mTA and pTA levels more than other monoamines. PLZ levels were least affected by the drug treatments, and that [2H2]-PLZ increased TRA, mTA and pTA levels more than other monoamines.
SEROTONIN ALTERS THE I-V CURVE AND THE EFFECTS OF DEPOLARIZING PREPULSES IN THE CYCLIC AMP-DEPENDENT CURRENT IN NEURONS OF THE WOLLUCS IN GUINEA PIG POSTERIOR CALLOSUMS. R.C. Huang and C.N. Gilbert, Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

In identified dorsal raphe neurons intracellular isocitrotransfection of cyclic AMP induced an inward current recorded under current clamp. Stepwise substitution of Na+ by tetraethylammonium caused a slow refrectory period, indicating a Na+ dependent current. In contrast to the activation of the current by depolarization in other identified serotonergic neurons of the guinea pig (Silveira & Green, this meeting), the I-V relation of the cAMP-stimulated current is the pedal neuron was approximately flat from -70 to -40 mV and rapidly decreased at more positive voltages. Conditioning pulses of depolarization and spiking caused transient partial suppression of cAMP response amplitude, which recovered over several minutes.

Both application of 10 nM serotonin invariably induced a large and sustained inward current. Concomitantly, the I-V relation of the cAMP-stimulated Na+ current was altered; response amplitudes at holding voltages greater than -30 mV were reduced in both amplitude and duration. Changes in the curve relating cAMP injection current to the Na+ current response suggest serotonin acts in part by increasing endogenous levels of cAMP.

Serotonin also acted to reverse the suppressive effect of conditioning depolarization. In the presence of serotonin, the cAMP responses were either unaffected by depolarizing prepulses or, more often, were potentiated. These data show that serotonin alters the responsiveness of these neurons to cAMP as well as the action of membrane potential on the cAMP-dependent ion channels.


Extracellular single unit recordings were obtained from superfused, 300 micron slices of rat midbrain. Because few 5-HT neurons in the dorsal raphe are spontaneously active in vivo, phencyclidine (10 μM) was added to the superfusate to maintain a basal firing rate. 5-HT dorsal raphe units were identified by their slow (0.25-5.0/sec), steady firing rate, and by an inhibitory response to 8-OHDPAT or 5-HT application. The effects of imipramine administered alone in the superfuse and imipramine potentiation of a minimally effective concentration of 5-HT (0.1 μM, mean 10 Hz inhibition) were studied. Imipramine (concentration range 0.5-25 μM) administered alone inhibited the firing rate of 5-HT neurons in a dose dependent manner (IC50 = 5.8 μM). The IC50 for imipramine enhancement of the effects of 0.1 μM 5-HT was 5.0 μM. The imipramine effects were blocked by 1-10 μM 1-(p-chlorophenyl)-2-piperazine, a 5-HTA antagonist. Fluoxetine suppressed the firing rate with an estimated IC50 of 2-4 μM. These results demonstrate that 5-HT dorsal raphe neurons respond to administration of 5-HT uptake inhibitors in the same manner in vivo as they do in vitro. Thus, j uplex single unit recording from the dorsal raphe means of obtaining a physiological measure of the potency of 5-HT reuptake blocking agents.
Numerous ligands which bind to 5-HT, receptors demonstrate agonist actions at dorsal raphe (DR) 5-HT autoreceptors, causing inhibition of firing of DR serotonergic neurons. Two 5-serotonin receptor blockers, pindolol (Sprinz et al., Karger, 1986, 128-295-298), and pindolol (Geelhoed and VanderMaan, Soc. Neurosci. Abstr. 1987) were used as well, antagonizing the effects of 5-HT agonists on DR-5-HT neurons. The compound BMY 7378 binds with high affinity to 5-HT, sites in the hippocampus (IC50=100-150 nM), but does not block the suppression of firing of DR neurons by 8-OH-DPAT in the DR, and also the hippocampus (Chaput de Montigny, Soc. Neurosci. Abst., 1987). Present studies investigated further possible agonist and antagonist properties of BMY 7378 in the rat DR, using in vivo and in vitro electrophysiological preparations.

Standard techniques were used for extracellular single-unit recordings from rat DR-5-HT neurons. For in vitro studies, test drugs were administered i.v. or by microinjection to chronically hydrated anesthetized rats. For in vitro studies, rats were anesthetized with chloral hydrate, allowed to breathe 100% O2 for 5 min, and then 400 μm thick Vibratome brain slices containing the DR nucleus were prepared. Superfusion artificial CSF containing in mM: NaCl, 110; KCl, 5.0; CaCl2, 1.25; MgSO4, 1.25; NaHCO3, 24; NaH2P04, 1.25; d-glucose, 10.0. Phenylephrine (PE, 10 μM) was present to provide increased neuronal activity. GEPs were applied by iontophoresis with chloral hydrate and allowed to breathe 100% O2 for at least 5 minutes. Frontal sections were cut through the DR nucleus and placed in a chamber through which 95% O2, 5% CO2 flowed. The slices were bathed in continuously flowing artificial CSF containing in mM: NaCl, 120; KCl, 5.0; CaCl2, 1.25; MgSO4, 1.25; NaHCO3, 24; NaH2P04, 1.25; d-glucose, 10.0.

In the Brain slice experiments, BMY 7378 produced inhibition of firing of DR neurons (ED50=0.03 mg/kg), as did microinjections of buspirone. In the in vitro electrophysiological study of BMY 7378 was adjusted to produce only partial inhibition of firing, this usually resulted in antagonism of the inhibitory effects of iontophoretically applied ipsapirone (as a 5-HT, ligand), and in some cases, of 5-HT as well. Similarly, in the Brain slice experiments, BMY 7378 produced inhibition of firing of 5-HT DR neurons (IC50=100-150 nM). If this inhibition was due to an agonist effect on 5-HT, receptors (as opposed to a block, for example, of the 5-HT, antagonist action), the compound BMY 7378 blocked the inhibition of firing produced by BMY 7378. Finally, when conditions were adjusted so that BMY 7378 did not produce complete inhibition of firing of DR neurons (BMY 7378 = 200 μM; PE = 10 μM), BMY 7378 antagonized the actions of 5-HT, agonist gepirone (p<0.001). Therefore, because of the combination of agonist and antagonist actions displayed by this compound, it is concluded that BMY 7378 functions as a partial 5-HT, agonist and antagonist.

Buspirone and 8-hydroxy-DPAT (DPAT), both of which bind to 5-HT, receptors, depress 5-HT neurons in the dorsal raphe (DR) where 5-HT, receptors are most abundant. To confirm that the effect is mediated at 5-HT, receptors, one needs to show that it can be reversed by a 5-HT, agonist. Spiperone binds to 5-HT, and 5-HT, receptors, and has been used as a tool for testing these effects on DPAT and buspirone in the DR. Action potentials of single 5-HT neurons were identified by large positive-negative voltages and slow (1-2 m/s), regular firing rates recorded from microelectrodes located in DR (Aghajanian et al., J PET 159;178, 1977), DPAT (ED50 = 1.7 μg/kg) and buspirone (ED50 = 15 μg/kg) dramatically depressed firing rates of 5-HT neurons. Spiperone, 1.0 mg/kg i.v., completely reversed the effects of 5-HT, agonists and occasionally increased rates above controls. Lower spiperone doses were weaker, and higher doses did not increase the effect and occasionally depressed 5-HT, cells. Spiperone's actions were not due to antagonism of 5-HT, receptors since L73387, a selective 5-HT, antagonist, was ineffective. Since spiperone is a D2 antagonist, studies were undertaken to determine if DPAT or buspirone had effects on D2 systems. Substantia nigra (SNPC) DA neurons were identified by large, duration-action potential actions with firing rates of 3-8 spikes/sec recorded from electrodes located in SNPC (Breunig et al., J PET 135;560, 1973). Doses of DPAT that silenced DR cells did not affect DA firing rates, nor did it reverse amphetamine depressions of DA neurons. Thats is, there is no interaction between DA and 5-HT systems. Higher DPAT doses (1 mg/kg) reversed amphetamine-induced depressions of DA neurons, which antagonizes D2 receptors, reduced amphetamine-induced release of DA in DA neurons, with a potency for reversing amphetamine depressions (10 μg/kg) similar to that for depressing 5-HT neurons (15 μg/kg). Thus, in the overall results, DPAT selectively blocks 5-HT, depression of 5-HT neurons. Since neither D2 antagonist nor 5-HT, antagonists reverse the depression of 5-HT neurons since these 5-HT, antagonist is unaffected, it is concluded that BMY 7378 reverses this effect by its action as an antagonist of 5-HT, receptors.
459.9 ELECTROPHYSIOLOGICAL RESPONSES OF HIPPOCAMPAL PYRAMIDAL CELLS TO SEROTONIN 5-HT1A AND 5-HT1B SELECTIVE AGONISTS: A COMPARATIVE STUDY WITH DORSAL RAPHE NEURONS. L-J. Makara and G.K. Aghajanian. Dept. of Psychiatry, Yale University Sch. of Med., New Haven, CT 06508.

The recently described 5-HT1-binding site subtypes (5-HT1A, 5-HT1B, 5-HT1C) are characterized by a marked regional distribution and binding densities in rat brain (Paxinos, Acad. Res. 76: 205, 1985). In particular, 5-HT1A sites are concentrated in the CA1 hippocampal pyramidal cell layer and in the hippocampal dentate gyrus. Consistent with these binding data, selective 5-HT1A agonists closely mimic the inhibitory effects of 5-HT on dorsal raphe cell firing whereas 5-HT1B agonists are comparatively much weaker. Furthermore, in intracellular recordings, the maximal hyperpolarizing effect of 5-HT1A agonists is substantially greater than that of 5-HT itself (Sprague & Aghajanian, Science 238: 1, 1987). A systematic examination of the effects of 5-HT1A and 5-HT1B selective agonists on spontaneously firing CA1 hippocampal pyramidal cells has not been reported previously.

Intracerebroventricular application of 5-HT reversibly suppressed CA1 pyramidal cell firing within the same current–dose range as that observed in the dorsal raphe. In contrast to the dorsal raphe, however, neither the 5-HT1A nor 5-HT1B agonists displayed potencies comparable to 5-HT when directly applied to the same cells: 8-OH-DPAT, IPSP; TXVQ 7821 and LY 165163 (PAPP), and two 5-HT1B agonists, selective agonists on spontaneously firing CA1 hippocampal pyramidal cells were not reported previously.

Intracellular recordings from CA1 pyramidal cells were made from unanesthetized rats tranquilized to produce a low cerebrospinal fluid. Compounds were applied by microiontophoresis and included three 5-HT1A agonists, 8-OH-DPAT, ipsapirone (IPSA; TXVQ 7821) and LY 165163 (PAPP), and two 5-HT1B agonists, selective agonists on spontaneously firing CA1 hippocampal pyramidal cells not previously exposed to 5-HT. Subsequent 5-HT applications onto the same neuron cause a hyperpolarization of sites are concentrated in the CA1 hippocampal pyramidal cell layer and in the hippocampal dentate gyrus. Consistent with these binding data, selective 5-HT1A agonists closely mimic the inhibitory effects of 5-HT on dorsal raphe cell firing whereas 5-HT1B agonists are comparatively much weaker. Furthermore, in intracellular recordings, the maximal hyperpolarizing effect of 5-HT1A agonists is substantially greater than that of 5-HT itself (Sprague & Aghajanian, Science 238: 1, 1987). A systematic examination of the effects of 5-HT1A and 5-HT1B selective agonists on spontaneously firing CA1 hippocampal pyramidal cells has not been reported previously.

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459.13 THE EFFECT OF LYSEIC ACID DIETHYLAMIDE ON SEROTONERGIC ELECTROPHYSIOLOGICAL RESPONSES IN RAT HIPPOCAMPAL SLICES. N.P. Clarke, S.G. Beck, and J. Goldfarb. Department of Pharmacology, Mount Sinai School of Medicine, CUNY, New York, NY 10029.

The superfusion of rat dorsal hippocampal slices with serotonin (5-HT) elicits at least two changes in the CA1 population spike evoked by stimulation of the stratum radiatum. The predominant effect, mediated by the 5-HT_{1A} receptor, is a rapid and reversible decrease in population spike amplitude (PSA). This decrease in the PSA is not observed if both drugs were administered concurrently. Apparently non-hallucinogenic antagonist, 2-bromo-LSD (BOL; 1-10 uM) also decreased the PSA but was less potent than LSD. The presence of spiperone (100 nM; 500 times its IC_{50} at the 5-HT_{1A} site), 5-HT (1-10 uM) produces only an increase in PSA. Even under these conditions, LSD (0.1-100 uM) did not increase the PSA. Moreover, LSD did not appear to alter the increase produced by concurrent administration of 5-HT (3-10 uM). Based on these results we suggest that LSD is an agonist at the 5-HT_{1A} receptor that mediates the decrease in PSA in rat hippocampal slices but LSD does not interact with the unclassified 5-HT receptor that mediates the increase in PSA. (Supported by grants DA-01875, DA-07315 and MH-41917.)


Serotonin (5-HT) reduces the width of tetrodotoxin-broadened (TEA) action potentials in frog dorsal root ganglion cells, presumably by reducing Ca influx (J. Neurosci., 6:620, 1986). The putative 5-HT_{2} agonist 8-DH dihydropipaminetetralin (DPAT) widens these action potentials (Marsalec et al., 1992). However, better understanding the receptors involved, serotonin antagonists were tested for their effects on the TEA-evoked serotonin induced increase in cell size. The superfusion of frog DRG cells with 5-HT in the presence of TEA evoked serotonin induced action potential duration in a dose-dependent manner. This effect was antagonized by methiothepin (MTP); the putative 5-HT_{1A} antagonist spiperone (SPIP) at 10 uM also reduced the narrowing response of 5-HT.

**Table:**

<table>
<thead>
<tr>
<th>Serotonin Conc.</th>
<th>% Control (No MTP)</th>
<th>% Control (2uM MTP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>86±6 (n=9)</td>
<td>92±2 (n=4)</td>
</tr>
<tr>
<td>1</td>
<td>86±6 (n=9)</td>
<td>93±2 (n=7)</td>
</tr>
<tr>
<td>10</td>
<td>49±7 (n=6)</td>
<td>79±4 (n=5)</td>
</tr>
</tbody>
</table>

This blocking effect persisted after one hour of washout and appeared to be non-competitive in that it could not be overcome with 100 uM 5-HT.

459.15 AGE-DEPENDENT CHANGES IN SEROTONIN DORSAL RAPHE NERVE ENDINGS. ELECTROPHYSIOLOGICAL ASSESSMENT IN FISHER 344 RATS. J.M. Lakoski and C.G. Richard. Department of Pharmacology and Toxicology, Univ. of Texas Medical Branch, Galveston, TX 77550.

Alterations in both hormone and neurotransmitter systems have been identified to underlie a variety of age-related changes in neuroendocrine function, including the decline of female reproductive function. Specific age-related changes in central serotonin (5-HT)-containing neuron systems in the aging female rat have been identified from neurochemical and behavioral studies. However, studies of cellular physiological events which may mediate age-related changes in reproductive function, particularly with respect to 5-HT neurons, are lacking. Therefore, as a prelude to studies evaluating age-related changes in sensitivity to microiontophoretically applied 5-HT agonists in the dorsal raphe nucleus (DRN), we have evaluated whether spontaneous 5-HT DRN cell firing is altered in the aging female rat.

Intact 10 mo and 20 mo old Fisher 344 rats were anesthetized with chloral hydrate and utilized for extracellular recording studies of 5-HT neurons in the DRN. Cycling young (3-4 mo), middle-aged (11-12 mo) and acyclic (11 mo) females overanesthetized at least one week prior to recording were also utilized to provide a constant level of circulating plasma estradiol comparable to that of the intact aged rats. Population sampling of spontaneously active 5-HT neurons in the DRN revealed no significant differences in the number of cells encountered (3 passes/animal) with respect to age. However, a significant age-related decline in the average firing rates of 5-HT cell sampled was apparent: 3 mo (10.36±0.82 sp/sec, n=61), 11 mo (8.76±0.64 sp/sec, n=44), 18 mo (7.29±0.57 sp/sec, n=75), and 20 mo (6.90±0.84 sp/sec, n=21) groups. Significant declines in the range of firing rates observed with respect to age were identified by statistical comparison (Mann-Whitney U) of rankings between age groups.

These data have identified specific age-related changes in 5HT cell function in the DRN that may underlie neuroendocrine events associated with female reproductive aging. Studies are presently underway to determine whether the pharmacologic profile of DRN 5-HT autoregulatory mechanisms have developed as a result of chronic cocaine administration (i.p.).

459.16 ENHANCED INHIBITORY RESPONSES OF SEROTONIN NEURONS IN THE DORSAL RAPHE NUCLEUS (DRN) AFTER REPEATED COCAINE EXPOSURE. F.D. Cunningham, E.K. Asprodinis, N.B. Barnard C.G. Richard*, and J.M. Lakoski. Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77550.

A potent stereoselective inhibitory effect of cocaine on serotonin (5-HT) cell firing in the DRN has been described after both systemic and microiontophoretic application. We investigated the effects of cocaine on both 5-HT cell populations in the DRN. After microiontophoretic application of cocaine, we observed a significant reduction in the response of 5-HT neurons recorded in the rat DRN after two regimens of chronic cocaine administration (i.p.): (1) 1.33±0.28 μg/kg once daily for 10 days; (2) 15 mg/kg/injection twice daily for 7 days. After either regimen of repeated cocaine or saline injections, we assessed the ability of cocaine (0.125-8.0 mg/kg, i.v.) or the 5-HT autoreceptor agonist 8-hydroxy-2-(di-n-propyl-amino)-tetralin (8-OHDPAT; 0.01-20 μg/kg, i.v.) to depress the firing rate of 5-HT neurons recorded extracellularly in the DRN. Cocaine recordings were conducted within 24-48 hours after the final treatment injection. The daily 10 mg/kg schedule did not significantly alter the ability of cocaine to inhibit the spontaneous activity of 5-HT neurons. In contrast, following the twice daily cocaine (15 mg/kg) treatment, the dose-response curves for both cocaine and 8-OHDPAT inhibitory responses were shifted 8-fold to the left in the 10 mg/kg females, and females overanesthetized at least one week prior to recording were also utilized to provide a constant level of circulating plasma estradiol comparable to that of the intact aged rats. Population sampling of spontaneously active 5-HT neurons in the DRN revealed no significant differences in the number of cells encountered (3 passes/animal) with respect to age. However, a significant age-related decline in the average firing rates of 5-HT cell sampled was apparent: 3 mo (10.36±0.82 sp/sec, n=61), 11 mo (8.76±0.64 sp/sec, n=44), 18 mo (7.29±0.57 sp/sec, n=75), and 20 mo (6.90±0.84 sp/sec, n=21) groups. Significant declines in the range of firing rates observed with respect to age were identified by statistical comparison (Mann-Whitney U) of rankings between age groups.

Recent work has suggested that noradrenaline (NA) may, in part, mediate the central effects of endogenous trace amines-\(\alpha\)-phenethylamine (PE) and it has been demonstrated that PE causes an increase in responses to iontophoretically applied NA. The present experiments were carried out to determine if the two amines mediate the same post-synaptic mechanism of this interaction.

Mice weighing 20-25 g were subject to one of the following pretreatments; none (control animals), reserpine (10 mg/kg, i.p., 24 hours) or vehicle (20% ascorbic acid, 2 ml/kg), \(\alpha\)-methyl-phe­nylalanine (\(\alpha\)-MPT, 500 mg/kg, i.p., 24 hours) or vehicle alone. PE and NA were iontophoretically applied to neurons in the cerebral cortex and the hippocampus, and iontophoresis circuits were used. Spontaneously active units were recorded 400-1200 \(\mu\)m below the pial surface.

In control animals 81% of the neurons responded to NA, and 65% to PE. Small currents of PE (6-8 nA) increased responses to NA to between 155% and 360% of control response size, without affecting the baseline firing rate, or responses to GABA or 5-HT. propranolol (PRO) stereoselectively binds to 5-HT1A receptors and HT2 antagonist spiperone are somewhat effective (Exp. Neurol. 1987 98:37). It has been shown that the \(\alpha\)-MPT pretreatment blocks the response to NA effectively antagonised 5-HT mediated inhibition in 6 of 8 neurons (75%) however only 25% demonstrated reasonable recovery of 5-HT effects. Average reduction for these 2 cells was 51% (-) PRO also antagonized inhibition elicited by 8-0H-DPAT in 7 out of 8 cells (72%) 8-0H-DPAT demonstrated recovery after PRO. Average antagonism of 8-0H-DPAT meditated inhibition was 31% (4) PRO reduced inhibition to 5-HT in 2 out of 8 cells (25%) and to 8-0H-DPAT suppression of PC firing. Qualitatively, (-) PRO may prove to be more effective against 8-0H-DPAT than against inhibition by 5-HT (8-0H-DPAT 5/9, 8-0H-DPAT 2/8, 23%). Moreover, inhibition of PCs by 5-HT may involve mechanisms in addition to 5-HT1A receptors. (Supported by NIH Grant ROI NS 19296)


Previous attempts to antagonize serotonin (5-HT) mediated inhibition of cerebellar Purkinje cells (PCs) have been relatively unsuccessful (Exp. Br. Res. 56:50, 1984) with the exception that the chlorides ionophore antibiotic picrotoxin (Lee, M. et al., Exp. Neurol, in press) significantly by continuous application of the chloride ionophore antagonist picrotoxin (Lee, M. et al., Exp. Neurol, in press). We have now shown that iontophoretic applications of 5-HT receptor agonists to PCs superfused with 8-0H-DPAT suppression of PC firing. Qualitatively, (-) PRO may prove to be more effective against 8-0H-DPAT than against inhibition by 5-HT (8-0H-DPAT 5/9, 8-0H-DPAT 2/8, 23%). Moreover, inhibition of PCs by 5-HT may involve mechanisms in addition to 5-HT1A receptors. (Supported by NIH Grant ROI NS 19296)


In previous reports (Darrow, et al., Soc. Neurosci. Abst. 16: 155, 1986) we have shown that cerebellar PC spontaneous firing rate is decreased by iontophoretically applied 5-HT and (-) PRO stereoselectively binds to 5-HT1A receptors. (Supported by NIH Grant ROI NS 19296)


In previous studies, we have found that inhibitory effects of iontophoretic application of serotonin (5-HT) on cerebellar Purkinje cells (PCs) were antagonised significantly by the 5-HT1A receptor agonist 8-0H-DPAT (Strahlendorf, J. et al., submitted). Since our previous studies indicate that (-) PRO is more effective than (+) PRO in antagonizing 5-HT and 8-0H-DPAT suppression of PC firing. Qualitatively, (-) PRO may prove to be more effective against 8-0H-DPAT than against inhibition by 5-HT (8-0H-DPAT 5/9, 8-0H-DPAT 2/8, 23%). Moreover, inhibition of PCs by 5-HT may involve mechanisms in addition to 5-HT1A receptors. (Supported by NIH Grant ROI NS 19296)
640.1 SOMATOSTATIN'S EFFECT ON MUSCULAR RECEPTOR-LINKED PHOSPHOINOSITIDETurnover in the Brain. S. Kito, R. Miyoshi and Shinichiro. Third Department*, Internal Medicine, Hiroshima University School of Medicine, Hiroshima 734, Japan.

In our previous studies, it was noticed that somatostatin affected oxtremore binding in the rat hippocampus whose muscarinic acetylcholine receptors were more dominantly of the M1 type. It has been proposed that central M1 receptors are strongly linked to phosphatidylinositol (PI) turnover. Based on this fact, the authors investigated the effect of somatostatin on cerebral-stimulated PI turnover in vitro. Using this method, somatostatin turned out to be more than the additive effect. On the other hand, protein kinase C activator or inhibitor modified this somatostatin's effect. H-7, a protein kinase C inhibitor, only increased carbamyl-induced accumulation of [3H]-IP by itself, but also exaggerated somatostatin's augmented effect to the extent more than the additive effect. On the other hand, 1-octanol-2-acetyl-glycerol inhibited carbamyl-induced [3H]-IP accumulation with the preservation of the somatostatin's enhancing effect. It means that the existence of a feedback mechanism between protein kinase C and somatostatin have been evidenced. The experiments support the fact that PI turnover is also related with GTP binding protein. In our experimental system, GTP-activated protein (IAP) intraventricularly injected 4 days prior to prepare hippocampal slices. It was noticed that somatostatin's effect on carbachol-stimulated [3H]-IP accumulation was enhanced. To analyse these results more precisely, the time course of the carbachol-stimulated accumulation of [3H]-inositol phosphates was studied and we observed that both IP3 and IP2 reached peak values much slower (10 minutes) in the cortex than in other non-neuronal tissues. Somatostatin's modulating effects on M1 receptors were further investigated taking this kinetic characteristics into consideration.


We have previously shown that VIP stimulates somatostatin release from rat cerebral and diencephalic cells in gla-free cultures (Brain Res., 336:67, 1985). This study we have investigated the mechanism involved in this effect. We have shown that VIP stimulates somatostatin release from rat cerebral cortical and diencephalic cells in gla-free cultures. An early (2 days in culture) exposure of cells to cyto­line (40 μg/ml) did not alter the VIP effectiveness of this day of culture, a highly purified preparation of neurons as revealed by a weak (5%) immunohistochemical staining for glial fibrillary acid protein (GFAP) and vimentin, two markers of astrocytes. Under these conditions when VIP induces somatostatin release from cerebral cortical and diencephalic cells, the peptide also causes large increases in intracellular cyclic AMP. Both the release of somatostatin and the increase in cyclic AMP elicited by VIP are time and dose-dependent and not modified by removal or modulation of cyclic AMP accumulation. These results suggest that cyclic AMP might not be the second messenger of VIP effect on the somatostatin secretion process and indicate that VIP-stimulation of cyclic AMP formation and VIP-stimulation of somatostatin release are two phenomena which could be dissociated. The data presented here indicate that VIP stimulates somatostatin release and increase intracellular cyclic AMP in a dose-dependent manner. We then evaluated the effects of a phorbol ester (Phorbol 12-myristate 13-acetate) on somatostatin's effect. In contrast to the known action of the phospholipid pathway, the absence of VIP, PMA alone elicited a dose-dependent increase in cyclic AMP release. These data taken together indicate that cyclic AMP formation is not a prerequisite condition for somatostatin release, and cyclic AMP accumulation in a dose-dependent manner. PMA also stimulates somatostatin release and does not inhibit activation by PMA that is obtained at saturating concentrations of VIP. Supported by GSAU from the CNRS (UA 1197) and from the INSERM (n° 584603).

640.3 EFFECTS OF STEROID ACETONIDES ON THE INCREASE IN cAMP-ADRENORCEPTOR BINDING CAUSED BY LESIONS OF CENTRAL 5-HT NEURONS. C.A. Stockmeier, D. Fox* and K.J. Kellar. Dept. of Pharmacology, Georgetown Univ. Sch. of Medicine, Washington, DC 20007.

Selective lesions of central 5-HT neurons increase the number of beta-adrenergic receptors in rat frontal cortex and hippocampus (Brain Res. 230:123, 1982). We have measured the effects of 5'-nucleotidase (NnH) on beta-receptor binding following 5,7-dihydroxytryptamine (5,7-DHT)-induced lesions of 5-HT neurons. In addition, the in vitro affinity of L-isoproterenol (L-Iso) for the beta-receptor in cortex was determined in control and lesioned rats. Anesthetized rats were pretreated with haloperidol and 5,7-DHT or saline was injected into both the dorsal and median raphe nuclei (7ug free base). Two weeks after the ventriculal lesion and 7 weeks after the lesion the content of 5-HT was reduced by 95% in the frontal cortex while the content of norepinephrine was not significantly affected.

The ventricular lesion resulted in a doubling of [3H]dihydroalpranolol ([3H]DA) binding to beta receptors in the hippocampus. The lesion-induced increase in binding was completely prevented by 100 μM Gpp(NH)p in vitro, while binding in control tissues was unaffected by Gpp(NH)p. Specific binding was defined with either 200 μM L-Iso or 10 μM di-propranolol. The lesion procedure produced a 74% increase in [3H]DHA binding in the hippocampus and no change in the ventricle. The enhancement in thalamus was enhanced 48% by the raphe lesion, and again the increase in binding was prevented by Gpp(NH)p. These data suggest that 5-HT lesion affects the number of beta-adrenergic receptors with the raphe lesion mechanism, with regard to their effect on cAMP accumulation. For this study, rat cerebral cortical slices (250 μm x 260 μm) were preincubated (60 min) with [3H]-adenine, after which BAC (100 μM) or phorbol 12-myristate, 13-acetate (PMA; 10 μM) was added and the incubation continued for 10 - 15 min. The slices were then exposed to ISO for 10 min, the reaction terminated, and the isoprenaline (ISO) binding sites are not significantly affected.
Layer V neurons from cat sensorimotor cortex were studied in an in vitro slice preparation using intracellular recording and single-electrode voltage-clamp (SEVC) techniques to modify the modulation of repetitive firing behavior and subsequent afterhyperpolarizations (AHPs) by neurotransmitters. Following adequate stimulation (sustained repetitive firing of the SEVC step) these neurons exhibited a long-lasting AHP (sAHP) lasting 3-5 s. Both norepinephrine (NE) and muscarine selectively reduced this sAHP and the underlying ionic currents in a dose-dependent manner. The NE effect was mediated via p1-adrenoceptors. The effects of NE and muscarine added at low doses but occluded at higher doses, suggesting convergence of action at some point after binding to these receptors. Consistent with activation of the adenylyl cyclase system, the NE effect was mimicked by extracellular application of dibutyryl-cAMP, 8-bromo-cAMP, or forskolin. In platelets, SQ22536 (Squibb) inhibits adenylate cyclase activity (Harris et al. J. Cycl. Nuc. Res. 5:125). Preliminary data suggest that SQ22536 can prevent NE from decreasing the sAHP. SQ22536 does not prevent muscarine from reducing the sAHP, suggesting that muscarine does not act via the adenylyl cyclase system, but rather through another pathway. We hypothesize that muscarine acts via the phospholipidinositol cycle, with activation of protein kinase C mediating the reduction of the sAHP. Further, we predict that the convergence of the effects of NE and muscarine occurs at, or after the phosphorylation step. We are currently testing these hypotheses.

Supported by NIH grants NS16972, NS22410 and NS07007.

E.R. Squilliet and Sois supplied a gift of SQ22536.
40.10 CHOLINERGIC STIMULATION OF SOMATOSTATIN SECRETION BY CEREBRAL COR-TICAL CELLS IN VITRO. S. Halvorsen* and B.J. Robbins, Section of Neuroendocrinology, Yale University School of Medicine, New Haven CT 06510.

The coincidental loss of cholinergic and somatostatinergic (SS) markers from cortical regions of individuals with senile dementia of the Alzheimer's type led us to investigate possible interactions between these two systems. The observed short-lived elevation in cortical electrical activity in response to acetylecholine (ACh; PNAS 82:6344) could be due to local release of ACh. However, it is also possible that ACh could stimulate SS release from cortical SS interneurons.

Using primary monolayer cultures of rat cerebral cortical cells derived from 16 day-old embryos, we examined the changes in the rate of release of immunoreactive SS (IRS) in response to addition of cholinergic agents. These cultures have been shown to synthesize and secrete both SS-14 and SS-28 (Endo 113:574). Cultures were used after 18-20 days in vitro (DIV). Medium was replaced by a Krebs-Ringer bicarbonate buffer, which itself was replaced every 20 min. IRS released per 20 min incubation was measured by a sensitive and specific RIA. We found a rapid increase in IRS release up to 1.5 fold above baseline during the first incubation. This response returned to normal by 80 min despite the continued presence of ACh. Carbachol (C) also increased IRS release, with a maximum increase of 2.5 fold above baseline with 0.1 mM C. Neither Ach nor C had an effect on cortical cells which were only 11 DIV. The effects of C were blocked by equimolar pirenzepine, QNX, and atropine. The action of C was blocked by QNX, atropine, and tetrodotoxin. The failure of AF64 pretreatment or of acute exposure to QNX to block the action of C or atropine suggested that endogenous cholinergic neurons, if present, were not altering IRS secretion. We conclude that SS neurons have the capacity to respond to cholinergic signals and that the loss of both markers in Alzheimer's disease may be due, in part, to this putative interaction. Supported by NIMH grants AM36848 and ADRDA grant P50-UB-06-056.

40.61 GLOMUS VASCULAR, MOTOR AND PAIN REFLEXES AT SEGMENTAL LEVELS IN THE DESCENDING SPINAL CORD. V. Madelian, D.L. Martin, K. O'Connor* and W. Shain, University of Texas Health Science Center at San Antonio, TX 78284-7382.

Previous studies have shown that descending spinal cord noradrenergic (NE) and norepinephrine (NE) pathways influence cardiovascular, motor and pain reflexes at segmental levels. These pathways terminate at all spinal levels and anatomical studies have demonstrated a common localization of certain terminals in the gray. Starke and Montel (Neuropharm. 12:1073-80, 1973) established that NE of an inhibitory or excitatory action on terminal 5-HT fibers regulates the release of 5-HT. More recently these receptors have been characterized as alpha2 subtypes. In view of this, the purpose of the present study is to determine its effect on neural and 5-HT receptor regulation of K+-induced 5-HT release in rat spinal cord synaptosomes and tissue slices.

1. Spinal cord synaptosomes or tissue slices (300 μm) were loaded with [3H]-serotonin (0.5 μM final concentration) and placed in a perfusion chamber and perfused with oxygenated Krebs-Ringer buffer. Basal release was measured for 20 min in two minute fractions at which time K+ (15 mM) was added to the perfusion medium for 10 min. (J. Pharmacol. Exp. Ther. 240:1-10, 1986). When drugs were tested, they were present during the last 10 min of washing and throughout depolarization. The [3H]-5-HT present in each fraction was expressed as a percentage of total releasable [3H]-5-HT.

The addition of NE (1-1000 μM) reduced [3H]-5-HT release in a concentration-dependent manner. Control K+-stimulated release (1.58 ± 0.39%) was reduced 18% by 1 μM NE and 54% by 100 μM NE. The alpha2 receptor agonist clonidine also reduced [3H]-5-HT release in a concentration-dependent manner with 100 μM releasing 68% of the basal value (53% decrease, 7 fold, p<0.05). This effect was blocked by the alpha2 antagonist yohimbine. In addition, basal release of [3H]-5-HT was increased by the addition of yohimbine alone.

In order to determine if endogenous NE contributed to the regulation of 5-HT release, selective NE uptake inhibitors were included in the NE stimulation medium during K+-induced depolarization. Thus the endogenous NE released from tissue slices would be made available to regulate presynaptic 5-HT receptors. Tomoxetine selective NE uptake inhibitors, reduced the K+-induced release of [3H]-5-HT. Tomoxetine 1 μM reduced release 18% while 10 μM reduced release 4% (53% decrease, maximum effect). These results suggest that integration of NE and 5-HT terminal function in spinal cord could occur through NE influences on 5-HT presynaptic "heteroreceptors" system.

Supported by NINDS 14546.
460.13 ADAPTIVE CHANGES IN HEART CHOLINERGIC-ADRENERGIC INTERACTIONS FOLLOWING 6-HYDROXYDOPAMINE TREATMENT, W.Y. Chen* and L.C. Wince. Dept. Zoology, College of Veterinary Medicine and College of Osteopathic Medicine, Ohio University, Athens, OH 45701.

Heart function is controlled by a dual innervation by sympathetic (adrenergic) and parasympathetic (cholinergic) neurons. Changes in heart function are elicited by changes in the tonic activity of these two neuronal systems as they interact in a complex manner. Adrenergic (ADR) nerves release norepinephrine to increase heart contractile force, and cholinergic (CHOL) nerves release acetylcholine at heart ventricles, not primary to diminish ADR effects. The heart is known to adapt to changes in its neural input. Past research has focused largely upon adaptive phenomena in either the ADR or CHOL systems individually, but the relevance of such adaptive changes to ADR+CHOL interactions in the heart is uncertain. The present study was performed to examine adaptive phenomena in CHOL-ADR interactions in the control of heart function. Sprague-Dawley rats (180-220 g) were injected i.v. with two doses of 50 mg/kg at 24 h intervals and sacrificed 1, 2, or 3 weeks following the initial dose. Pulmonary nerves were dissected from the left ventricle, suspended in a tissue bath containing oxygenated Krebs-Henseleit buffer, and electrically stimulated to elicit isometric contractions. The isotropic potency of the beta antagonist isoproterenol (ISO) was studied in muscles from 6-OHDA treated and control rats. Supersensitivity to ISO was found at 1 and 2 weeks after 6-OHDA treatment as evidenced by 7.0 and 3.6 fold shifts, respectively, in the concentration-response curves. The ability of the muscarinic CHOL agonist carbachol (CARB) to attenuate the isotropic effects of ISO was also examined. In papillary muscles from control rats CARB (10 µM) reduced the isotropic effects of ISO as shown by a 2.2-fold shift of the concentration-response curve to the right. In contrast, CARB (10 µM) produced 9.1- and 9.6-fold shifts of the ISO concentration-response curve to the right at 1 and 2 weeks, respectively, after 6-OHDA treatment. We have also examined the ability of CARB to attenuate ISO-stimulated adenylate cyclase activity in ventricular homogenates. Preliminary results from these experiments indicate that CARB produces a greater shift to the right of the ISO concentration-response curve in homogenates from 6-OHDA treated animals at 2 weeks relative to controls. These results suggest that adaptive changes occur in the CHOL system after 6-OHDA treatment which increase the attenuation of the isotropic effects of ISO by CARB. Also, our preliminary data indicate that these findings may be related, in part, to an increased inhibition of ISO-stimulated adenylate cyclase activity by CARB. (Supported by Central Ohio Heart Chapter, AHA, Grant 87-41A and the Ohio Univ. Coll. of Osteopathic Medicine.)

460.14 EFFECT OF SYSTEMIC KAINIC ACID ON REGIONAL THH CONCENTRATIONS IN RAT CENTRAL NERVOUS SYSTEM, M.S. Kreider, B.L. Wolfinger* and L. Winkleman. Dept. of Psychiatry, Univ. of Penna., Philadelphia, PA 19104

Several studies have suggested that TRH levels in the CNS are influenced by the level of CNS activation. Hibernation and activation result in region-specific decreases in TRH concentrations. Repeated electroconvulsive shock and amygdala-kindling has been shown to result in elevations of TRH concentrations in limbic regions of the rat central nervous system. We investigated whether the production of limbic seizures by systemic administration of kainic acid would produce similar increases in TRH concentrations.

Male Sprague-Dawley rats (180-220 g) received injections of kainic acid, 12 mg/kg s.c., or saline. Rats were sacrificed 1, 3, or 7 days later. Brains were dissected into 10 regions, olfactory bulb, septum, corpus striatum, anterior cortex, brain stem, hypothalamus, posterior cortex, thalamus/midbrain, hippocampus and amygdala/piriform cortex. Each region was weighed, homogenized in phosphate buffered saline and extracted with methanol. Methanol extracts were dried, redissolved in 0.25% BSA/0.1 M sodium phosphate buffer, pH 7.6 and assayed for TRH content by radioimmunoassay.

Kainic acid administration resulted in very substantial increases in the concentration of TRH in anterior cortex, posterior cortex, hippocampus and amygdala/piriform cortex. Significant elevations were measured at all time points examined. Maximum increases of 300% for anterior cortex, 2700% for posterior cortex, 1600% for hippocampus and 2000 fold for amygdala/piriform cortex were observed 3 days following administration of kainic acid. At 4 and 7 days, TRH concentrations of kainic acid treated rats were significantly higher than controls but were progressively lower compared to 3 day kainic acid treated rats. TRH concentrations in the remaining CNS regions were unaffected by kainic acid administration.

These results indicate that induction of limbic seizures by systemic administration of kainic acid is extremely effective in increasing TRH concentrations in limbic regions of the rat central nervous system. This may represent a useful model for investigations of the neurochemical systems involved in the regulation of TRH in the central nervous system.

460.15 GABAERGIC INNERVATION OF CORTICOTROPIN RELEASING FACTOR NEURONS IN THE RAT PARAVENTRICULAR NUCLEUS: AN ELECTRON MICROSCOPIC IMMUNOCYTOCHEMICAL STUDY. J.A. Dlachowska, Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

The paraventricular nucleus (PVN) with its corticotropin releasing factor (CRF) neurons has been implicated in the control of biochemical, physiological and behavioral responses to environmental changes. However, the neural mechanisms underlying such adaptive responses have been difficult to unravel and the anatomical circuitry remains largely undefined. Most recently, the CRF neurons of the PVN have been implicated in a number of clinical situations such as depression and anxiety, TRH high levels of plasma cortisol in these patients appears to be due to a defect in the regulation of CRF release which then results in hypersecretion. As a result, we have begun to analyze the various putative inhibitory inputs to these CRF neurons. This study reports the results of an anatomical analysis of GABA-containing terminals in relation to CRF neurons of the rat PVN.

The relationship of GABA terminals with CRF neurons was first examined by a double labeling immunocytochemical technique at the light microscopic level. Using an antisera directed against glacial acid decarboxylase (GAD), a gift from Dr. D. Schmeckel), the PVN of male Sprague Dawley rats was stained using avidin-rhodamine as a label. Within the fluorescence microscope, a dense plexus of GAD positive terminals was observed in close relation to CRF neurons of the PVN, have also examined the ability of CARB to attenuate ISO-stimulated adenylate cyclase activity in ventricular homogenates. Preliminary results from these experiments indicate that CARB produces a greater shift to the right of the ISO concentration-response curve in homogenates from 6-OHDA treated animals at 2 weeks relative to controls. These results suggest that adaptive changes occur in the CHOL system after 6-OHDA treatment which increase the attenuation of the isotropic effects of ISO by CARB. Also, our preliminary data indicate that these findings may be related, in part, to an increased inhibition of ISO-stimulated adenylate cyclase activity by CARB. (Supported by Central Ohio Heart Chapter, AHA, Grant 87-41A and the Ohio Univ. Coll. of Osteopathic Medicine.)

To study this putative interaction, a unique double labeling method was employed at the electron microscopic level. First, rats were perfused with an acrolein-parafomaldehyde mixture, vibratome sectioned and the tissue stained with acrolein, osmic acid (OSA), a gift from Dr. D. Schmeckel), the PVN of male Sprague Dawley rats was stained using avidin-TRH content by radioimmunoassay. Significant elevations were measured at all time points examined. Maximum increases of 300% for anterior cortex, 2700% for posterior cortex, 1600% for hippocampus and 2000 fold for amygdala/piriform cortex were observed 3 days following administration of kainic acid. At 4 and 7 days, TRH concentrations of kainic acid treated rats were significantly higher than controls but were progressively lower compared to 3 day kainic acid treated rats. TRH concentrations in the remaining CNS regions were unaffected by kainic acid administration.

These results indicate that induction of limbic seizures by systemic administration of kainic acid is extremely effective in increasing TRH concentrations in limbic regions of the rat central nervous system. This may represent a useful model for investigations of the neurochemical systems involved in the regulation of TRH in the central nervous system.
461.1 DIFFERENTIAL EXPRESSION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR a-SUBUNIT mRNAs IN RAT BRAIN. E. Wada1, J. W. Swanson1, J. J. Bouiller, K. Wada1, S. Heinemann1 and J. Patrick1. 1Neural Systems Laboratory, 2Howard Hughes Medical Institute and 3Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037.

We have identified three genes in the rat genome that code for nicotinic cholinergic receptors expressed in the brain. The genes encoding a-subsunits of the nicotinic acetylcholine receptor gene family have been designated alpha2, alpha3, and alpha4 (expressed in muscle) and alpha2, alpha3, and alpha4 (expressed in neurons). We have used in situ hybridization histochemistry to localize the sites of expression of these genes in the rat central nervous system. Because of the high homology in the protein coding regions of the cDNAs encoding these proteins we have used parts of the less conserved 3' untranslated sequences to make [35]S probes for in situ hybridization. Our initial results with these probes demonstrate clear differential expression of alpha2, alpha3 and alpha4 in the midbrain and the interpeduncular nucleus. Neuroanatomical data indicates that the medial habenula receives a cholinergic input from the basal forebrain and that cholinergic neurons in the habenula in turn project to the fasicus retroflexus to form synapses in the interpeduncular nucleus. Our results indicate that the alpha2 and alpha4 genes are expressed at high levels within cells of the medial habenula while alpha3 transcripts are undetectable in this nucleus. In contrast, the alpha2 and alpha3 genes are expressed in the interpeduncular nucleus. These results clearly show that there is differential expression of the alpha2, alpha3, and alpha4 genes in cholinergic neurons of two distinct but interconnected nuclei in the rat brain. Recent experiments demonstrate that neurons in the medial habenula contain functional nicotinic cholinergic receptors (McCormick and Price, 1987, J. Physiol. 341:655). These receptors may be encoded by the alpha2 and alpha3 genes. Likewise, nicotinic receptor mediated excitation has been observed in the interpeduncular nucleus (Brown, Fochery, and Hallwesi, 1983, J. Physiol. 341:655). These receptors may be encoded by the alpha2 and alpha3 genes. These results suggest that the alpha2, alpha3, and alpha4 genes code for a-subunits of three different nicotinic receptor systems expressed in the brain.

461.2 IMMUNOHISTOCHEMICAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS IV 1657

Between the abducens and trigeminal nuclei, is implicated in REM sleep. We have used parts of the less conserved 3' untranslated sequences to make [35]S probes for in situ hybridization. Five (S) probes demonstrate clear differential expression of alpha2, alpha3 and alpha4 in the medulla oblongata and the interpeduncular nucleus. Neuroanatomical data indicates that the medial habenula receives a cholinergic input from the basal forebrain and that cholinergic neurons in the habenula in turn project to the fasciculus retroflexus to form synapses in the interpeduncular nucleus. Our results indicate that the alpha2 and alpha4 genes are expressed at high levels within cells of the medial habenula while alpha3 transcripts are undetectable in this nucleus. In contrast, the alpha2 and alpha3 genes are expressed in the interpeduncular nucleus. These results clearly show that there is differential expression of the alpha2, alpha3, and alpha4 genes in cholinergic neurons of two distinct but interconnected nuclei in the rat brain. Recent experiments demonstrate that neurons in the medial habenula contain functional nicotinic cholinergic receptors (McCormick and Price, 1987, J. Physiol. 341:655). These receptors may be encoded by the alpha2 and alpha3 genes. Likewise, nicotinic receptor mediated excitation has been observed in the interpeduncular nucleus (Brown, Fochery, and Hallwesi, 1983, J. Physiol. 341:655). These receptors may be encoded by the alpha2 and alpha3 genes. These results suggest that the alpha2, alpha3, and alpha4 genes code for a-subunits of three different nicotinic receptor systems expressed in the brain.


The medial pontine reticular formation (mPRF), which lies between the abducens and trigeminal nuclei, is implicated in REM sleep. Evidence from both extracellular and intracellular studies indicates that mPRF neurons depolarize before REMS onset and maintain a tonic level of depolarization throughout REMS. The depolarization of some mPRF neurons, therefore, may recruit other reticular neurons so that when a sufficient number are depolarized and a threshold of excitation is reached then REMS is generated. We hypothesize that acetylcholine is the neurotransmitter responsible for initiating the early depolarization of neurons in the mPRF. Cholinergic agonists are the only class of compounds that rapidly depress the mPRF neurons in vitro and maintain a tonic level of depolarization in situ. We have used parts of the less conserved 3' untranslated sequences to make [35]S probes for in situ hybridization. Five (S) probes demonstrate clear differential expression of alpha2, alpha3 and alpha4 in the medulla oblongata and the interpeduncular nucleus. Neuroanatomical data indicates that the medial habenula receives a cholinergic input from the basal forebrain and that cholinergic neurons in the habenula in turn project to the fasciculus retroflexus to form synapses in the interpeduncular nucleus. Our results indicate that the alpha2 and alpha4 genes are expressed at high levels within cells of the medial habenula while alpha3 transcripts are undetectable in this nucleus. In contrast, the alpha2 and alpha3 genes are expressed in the interpeduncular nucleus. These results clearly show that there is differential expression of the alpha2, alpha3, and alpha4 genes in cholinergic neurons of two distinct but interconnected nuclei in the rat brain. Recent experiments demonstrate that neurons in the medial habenula contain functional nicotinic cholinergic receptors (McCormick and Price, 1987, J. Physiol. 341:655). These receptors may be encoded by the alpha2 and alpha3 genes. Likewise, nicotinic receptor mediated excitation has been observed in the interpeduncular nucleus (Brown, Fochery, and Hallwesi, 1983, J. Physiol. 341:655). These receptors may be encoded by the alpha2 and alpha3 genes. These results suggest that the alpha2, alpha3, and alpha4 genes code for a-subunits of three different nicotinic receptor systems expressed in the brain.

461.4 QUANTITATIVE AUTORADIOGRAPHIC ANALYSES OF SELECTIVE MUSCARINIC RECEPTOR AGONIST BINDING WITHIN REGIONS OF RAT BRAIN. W. Hoss, B.R. Elderbrook*, M.A. Price*, AND W.S. Master, Jr. Department of Medicinal Chemistry, College of Pharmacy, University of Toledo, Toledo OH 43606.

The binding of four muscarinic receptor agonists to regions of rat brain were examined through quantitative autoradiographic techniques. Oxtremorine, arecoline, pilocarpine and bethanechol were chosen on the basis of their different relative potencies and efficacies in muscarinic second messenger systems. Overall, the order of potency for inhibition of [3H]quinuclidinyl benzilate binding to rat brain slices was oxotremorine > pilocarpine > arecoline > bethanechol. Regional assays of agonist potency indicated that all agonists were more selective for brainstem and thalamic regions than for hippocampal and corticofugal regions. Thus all of the agonists were M4-selective. It appears that differences in binding do not explain the differences in abilities of the agonists to activate second messenger systems.

Of the four agonists examined, pilocarpine displayed the highest selectivity for M2 receptors in that IC50 values for pilocarpine were three-fold higher in the hippocampal and striatal regions (e.g., CA3: 40.6 ± 9.4 µM) than in thalamic and thalamic regions. Arecoline and bethanechol were 19- and 100-fold more selective for brainstem and thalamic regions. The ability for agonists to display curved Scatchard plots within the external layers of the cerebral cortex, hippocampus, septal nuclei, and corpus geniculatum laterale ventrale. Fibers lateral to the labelled cells in the posterior limbic cortex and midbrain. An adult chicken brain was immersion fixed with 4% paraformaldehyde in 0.1 sodium phosphate, pH 7.4. Paraaxial and sagittal silicosis, 60 µ thick, were cut on a microtome and pretreated with 0.1% hydrogen peroxide to destroy endogenous peroxidase. Free floating silicos were incubated overnight with primary antisera in dilutions of 1:3000 to 1:5000. An avidin-biotin-horseradish peroxidase staining procedure using diamobisnium chloride and cobalt enhancement was used to visualize the primary antisera.

Several areas of the midbrain were stained for choline acetyltransferase. Darkly stained cell bodies were seen in tectal layer III and the medial isthmal paraventricularis, semilunaris and spiriform laterales. Stained fibers appear throughout the tectum as well as in the nuclei isthmi magnocellularis, interstitialis and corpus geniculatum laterale ventrale. Fibers lateral to the labelled cells in the spiriform laterale are also stained. The cells stained in tectal layer III are radial neurons and their processes can be seen running in the tectal fiber layers of the optic tectum. The localization of choline acetyltransferase containing cells and fibers further defines areas of cholinergic transmission in the chicken midbrain. This, together with other data, will help in the localization of putative nicotinic receptor containing neurons and in distinguishing their possible functions as they are better characterized within different areas of the chicken midbrain. Supported by NIH Grant 5217574 to Y.A.C.

Previous work from this laboratory has evaluated the use of [3H]scopolamine in vivo measurement of muscarinic cholinergic receptors in the rat brain (Frey et al., J. Neurosci. 541, 1985). In these studies, the distributions of muscarinic receptor binding is extended to the study of human brains with the use of [11c]scopolamine and positron emission tomography (PET).

[3-methyl-11c]scopolamine was synthesized from norscopolamine by radiolabeling with 3-methyl-11cCl using a radioiodination method. Following injection into the radial artery catheter at intervals for determination of the scopolamine activity curve. After centrifugation, samples of the plasma were applied to liquid chromatographic columns (SEP-Pak C18 G) for separation of scopolamine from labeled metabolites prior to activity measurement in a gamma counter.

Results of the four studies were qualitatively similar. During the first minute, significant tracer concentrations were observed in major vascular structures (cerebral arteries, veins, and sinusoids). Within the first few minutes following injection, brain parenchymal tracer concentration increased at a rapid rate, followed by a significant reduction in tracer concentration in the cerebral white matter. Rates were 0.04-0.05, indicating extractions of ~10% of brain blood flow.

Estimates of the binding rate and distribution parameters were obtained by using a bi-specific rat monoclonal antibody against Substance P (SP). In the peripheral cut end was then placed on a small piece of paraffin wax, to prevent leakage. Following a survival period of 72 hours, the animals were perfused with 4% paraformaldehyde. The trigeminal ganglia was cut at the level of the origin of the superior laryngeal nerve, the vagus, to prevent leakage. The trigeminal ganglia was dissected out in the high resolution mode. Arterial blood samples were withdrawn from a radial artery catheter at intervals for determination of the scopolamine activity curve. After centrifugation, samples of the plasma were applied to liquid chromatographic columns (SEP-Pak C18) for separation of scopolamine from labeled metabolites prior to activity measurement in a gamma counter.

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461.9 THREE-DIMENSIONAL LOCALIZATION OF ^{125} I-NEUROTENSIN BINDING SITES IN THE RAT MEDULLA OBONSIUS USING DIGITIZED IMAGE PROCESSING. J.P. Wattiaux, G. Hejblum, M. Vial, W. Rothe, INSERM U55, 184 rue du Faubourg St Antoine, 75571 PARIS CEDEX 12, France.

We have recently shown that high densities of neuropeptide (NP) binding sites are present in both rat substantia nigra pars reticulata and ventral tegmental area (VTA). In those brain regions several lines of evidence suggest that NP binding sites are located on dopaminergic cell bodies and dendrites. The aim of the present work was to develop a computerized approach for three-dimensional representation of the localization of NP binding sites in both SN and VTA. We selected the entire structures in a three-dimensional space using 80 serial mesencophalic sections obtained from one rat brain, located within the limits of SN pars reticulata (SNr) and TH-immunoreactive analysis on USJ films as previously described (Baratte et al, Brain Res. 346: 375, 1985). A video camera coupled with an IMAX image analyzer was used to sequentially digitize the series of autoradiograms as well as the structure outline after respective alignment. The labeled regions were discriminated with a demisotropic threshold. We developed a three-dimensional reconstruction algorithm (based on voxel representation) which enabled us to visualize the inside of the reconstructed objects.

Because of several unavoidable alterations of the slice’s shape mainly during sectioning and mounting, the irregularities observed in the reconstructed 3-D shape for each brain were corrected with an original non-linear least squares three-dimensional filter. An opaque and three-dimensional picture with different shadings was then obtained at various orientations which simultaneously represented with one color both VTA and SN outlines, and with another one the labeled areas.

NP binding sites are observed in the zona incerta and their density increases when the SN pars compacts appears. Caudally, both dorsal and ventral labeled areas of the SN pars compacta describe a ring around the substantia nigra and ventral tegmental area (VTA). The latter is less intensively labeled and increases when the SN pars compacta appears. Caudally, both dorsal and ventral labeled areas. The former area shows a great number of labeled stripes which probably correspond to the reconstructed 3-D shape for each brain were corrected with an original non-linear least squares three-dimensional filter.


Cholecystokinin (CCK) binding sites were localized in the hippocampus, amygdala, and medial temporal cortices of macaque monkeys using the 125I receptor autoradiography. Binding sites were labeled with ^{125} I-CCK-8 and ^{125} I-CCK-33 and non-specific binding was assessed in the presence of 1 uM CCK-8. Comparison of autoradiograms with Nissl stained sections allowed precise correlation of autoradiographic grain distribution with cytoarchitectonic divisions. In the hippocampal formation a single dense band of label was observed over the granule cell layer and adjacent few millimeters of the molecular layer of the dentate gyrus. This heavy band of label stood out sharply from the very low density binding of both the polymorph and remaining molecular layers. Binding was similarly concentrated in the presubiculum, particularly over the densely packed pyramids, clearly distinguishing this region from the adjacent subiculum in which binding just exceeded background levels. Moderate to light label was observed in the hiru and stratum pyramidale of CA3 while remaining hippocampal layers showed minimal specific binding. CCK binding in the amygdala varied among nuclear subdivisions. The lateral, basomedial, endopiriform, and cortical nuclei showed dense CCK binding, while labeling was notably sparse in the central, medial, basolateral, and accessory basal nuclei. Variations in CCK binding in the medial temporal cortices showed close correspondence to cytoarchitectonic divisions. Thus, entorhinal cortex binding was concentrated in layers III, IV, and V, while, binding in area TH was more evenly distributed among layers. Area TP was distinguished from other regions by the emergence of a bimodal pattern of labeling in which binding sites were concentrated in layers II and IV.

The variation in CCK binding throughout the medial temporal lobe suggests that in this region CCK receptor localization is determined by factors other than diffusive influence on cellular elements. This influence may include modulation of anterior and posterior cortical regions, which are anatomically coincident with CCK binding in the lateral amygdaloid nucleus, the external granule cell layer of the presubiculum, and deep layers of the entorhinal cortex (Brog et al, 1986, Neurosci. 12: 719; Aggleton et al., 1980, Brain Res. 207: 347). These findings may also bear on recent evidence of CCK depletion in temporal lobe structures in schizophrenics (Ferrier et al., 1983 Life Sci. 33: 475) whose predominant 'negative' symptoms may be linked to a failure of integrative neural mechanisms in the frontal lobe dysfunction. Supported by MH38546 and NS22807.


The African lungfish (Protopterus) contains high concentrations of the neuropeptide releasing hormone (TRH) in the central nervous system, with concentrations in the telencephalon and diencephalon ranging from 5-10 fold higher than in comparable regions of rat brain. TRH produces potent effects on respiration in several species, and has also been shown to exert behavioral arousal in the CNS, we examined the localization, quantification, and characterization of TRH receptors in the CNS of the lungfish.

Nonspecific binding was assessed by addition of 1 uM unlabeled Ang II. Analysis of autoradiographic images confirmed the presence of specific, high affinity Ang II binding sites in the cell body region of the NG (Ka = 0.66 ± 0.08 nM) and the trunk of the VN (Ka = 0.83 ± 0.26 nM). After ligation, Ang II binding sites (Ka = 0.51 ± 0.08 nM) accumulated at LIG1 (on the side nearest the NG), revealing anterograde transport. To investigate possible transport of Ang II binding sites in the VN, quantitative in vitro receptor autoradiography was used to visualize binding after double ligation of the peripheral process of the cervical VN in 8 dogs. Under halothane anesthesia, one ligature (LIG1) was tied 0.2 - 0.5 cm from the NG and the second ligature (LIG2) was tied on the same nerve 1.0-1.5 cm from the NG. Twenty-four hours later, the dogs were perfused with phosphate buffered saline containing 0.04% formalin. The untouched and ligated VN were removed and frozen. Ang II binding was determined by in situ hybridization with 14-16 µM sections in sodium phosphate buffer containing dithiothreitol, EDTA, NaCl, MgCl₂, and 0.4% bovine serum albumin plus 0.01-0.16 nM ³H-Ang II. Nonspecific binding was assessed by addition of 1.3 nM unlabeled Ang II. Analysis of autoradiographic images confirmed the presence of specific, high affinity Ang II binding sites in the cell body region of the NG (Ka = 0.66 ± 0.08 nM) and the trunk of the VN (Ka = 0.83 ± 0.26 nM). After ligation, Ang II binding sites (Ka = 0.51 ± 0.08 nM) accumulated at LIG1 (on the side nearest the NG), revealing anterograde transport. Retrograde transport of Ang II binding sites was assessed by ligating 0.5 cm of a similar site further (Ka = 0.51 ± 0.08 nM) seen distal to LIG1. These data reveal the existence of a mechanism for the bidirectional axonal transport of Ang II binding sites in the cervical portion of the VN. Further studies are needed to determine whether Ang II binding site transport is present in afferent or efferent fibers, or a combination of both, and whether it occurs in association with bound peptide. These observations and new information about the ligand-binding mechanism of action of Ang II on cardiovascular and possibly other autonomic reflexes. (Supported in part by NHLBI Grant HL-8635 and the Weinerberger Foundation).
461.13 MATCHING ANGIOTENSIN WITH ITS RECEPTORS IN RAT BRAIN: IMMUNOHISTOCHEMISTRY AND LIGAND BINDING. B. Wallace Lind, Andrew M. Allen*, Fred A.O. Mendelsohn*, and Detlev Ganten*. The Falk Institute, La Jolla, CA 92037; The University of Melbourne, Australia; The University of Heidelberg, Germany.

Neuroanatomical approaches to understanding angiotensin in the brain address two fundamental questions: Where is the peptide produced and where does it act. To answer the first question, immunohistochemistry has been used to identify neuronal cell groups that contain angiotensin immunoreactivity. It is assumed that such cells synthesize the peptide and ship it out over different fiber pathways for use as a chemical messenger. Towards answering the second question, binding of labeled angiotensin (or any of its high-affinity analogues) has identified presumptive sites of action. It is assumed that at locations where binding occurs there are membrane-bound receptors that detect the presence of the ligand diffusing across a synapse. If angiotensin does indeed act according to these prevailing notions about the behavior of neurotransmitters in the brain, and if our techniques possess adequate fidelity and sensitivity, the location of immunohistochemically identified cell groups and fiber systems should form a complement to the distribution of binding sites. To put it another way, one would expect to find evidence of angiotensin receptors in the places where angiotensin-containing neurons send their axons.

The results of the present work establish that such a complementary relationship does exist in the rat brain. We have found at least thirty distinct locations where angiotensin-immunoreactive terminal fields overlap concentrations of binding sites. In some instances, for example in the bed nucleus of the stria terminalis and in the paraventricular of the hypothalamus, the co-distribution of terminals and receptors is concordant at the subnuclear level. In other cases, for example in the ventral subdivision of the lateral geniculate nucleus, the two techniques provide evidence for a hitherto unknown site of action of angiotensin. And in other cases, for example in the projection from the cerebral cortex to the layers of the piriform cortex, improvements in immunohistochemical staining have identified an angiotensin-containing pathway that was suggested by earlier binding work.

In conclusion, the overwhelming agreement between these two converging lines of evidence gives us a list of likely sites of action. The next steps are to identify the parent cell groups giving rise to the various terminal fields, and to clarify the different traffic resulting from the activation of the several receptor pools. Eventually, such an understanding of morphology will form the groundwork for an elucidation of the full range of functions observed by angiotensin in the brain.

461.14 PRESENCE OF RELAXIN-LIKE PEPTIDES IN RAT BRAIN. Charles Babbit, Helen Kado-Iwong*, Derf Lewis*, Geoff Tropean* and Bernard Maley*. Pharming Technologies, Inc, South San Francisco, California 94080, and Howard Florey Institute, Melbourne, Australia.

Relaxin is a polypeptide hormone found in blood and reproductive tissues of a wide array of nonmammalian and mammalian species. Relaxin acts by binding to specific neuronal cell groups that contain relaxin immunoreactivity. It is produced in the ovary and is a product of pregnancy. Relaxin is found in decidua, placenta and ovarian corpora lutea as well as testes, seminal plasma and prostate glands.

The physiological role of relaxin is poorly understood. Relaxin has effects on uterine activity, cervical dilation during late pregnancy, and sperm motility. More recently, relaxin has been shown to modulate myometrial and vasopressin release from hypophysial nerve terminals in vitro.

The only known source of relaxin in the adult rat is the corpora lutea of the female. We hypothesized that relaxin may be produced by other organs, specifically the brain.

Extracts from ovaries and brain from nonpregnant and pregnant (day 20) female rats, and also testes and brain from male rats were prepared and analyzed by Western blot. Relaxin-like immunoreactivity was detected in brains of the pregnant and nonpregnant rats but only in the ovaries of pregnant rats. In the male rat, relaxin like immunoreactivity was found in the brain and testes.

Northern blot hybridisation analysis of polyadenylated RNA from brains and ovaries of pregnant and nonpregnant rats using a probe encoding rat relaxin showed two bands one at 0.6 kb and a more predominant band at 1.8 kb. Analysis of mRNA for specific brain regions of female rats showed the presence of mRNA encoding relaxin rat relaxin in cortex, hippocampus, hypothalamus, striatum and cerebellum.

The above data suggest that relaxin-like peptides are not only produced by the ovary, but also by the brain. To our knowledge this is the first demonstration of the presence of relaxin in rat brain.


The number of neurons in the sacculus portion of the eighth nerve increases with fish size in adults of the teleost species Astromonotus ocellatus (Pope and Hoxter, Hearing Res, 1984). The source and location of the new neurons, however, are not known. To ascertain the source of the new cells, adult ocellatus, 5 cm in standard length, were injected with tritiated thymidine. Each fish received a total of four injections of 5 micromC/g/hw. The injections were separated in time by 1 week. Following the last injection the animals survived for 4-6 weeks. The eighth nerves were embedded in plastic (JB-4), sectioned at 4 microns and processed in a standard fashion for autoradiography.

Following this injection schedule no mature eighth nerve neurons were labeled with thymidine. However, examination of the sections revealed small clusters of cells that are clearly different in morphology from either eighth nerve neurons or glial cells. A few of the cells in these clusters were labeled with tritiated thymidine. The cells in the clusters are about 5 microns in diameter, show dark, dark, granular nuclear staining with Nissl stain, and have no detectable surrounding cytoplasm. Surrounding the cells are clusters with larger, pale staining nuclei, and very little or no cytoplasm. Surrounding these are cells that look like small eighth nerve neurons, but with no apparent processes. Based upon these observations we suggest that the clusters of small cells are the precursors to new eighth nerve neurons, and the surrounding cells are differentiating nerve neurons.

In a single aural (eighth) nerve there are 2 or 3 such clusters. The clusters are connected by a thin string of what appear to be similar types of cells. This string of cells also connect the aural clusters to similar clusters in the lagenar and utricular portions of the eighth nerve. The clusters vary in size, suggesting that the number of eighth nerve neurons produced may vary across the epithelium. Experiments designed to verify these cells as eighth nerve precursors are in progress. (Supported by NIH)


Accumulation of ventromedial hypothalamic (VMH) neurons has been previously shown to occur in the two groups of animals, estrogenic and non-estrogenic (MCG) (Chung et al., 1984, 1986). Since VMH synapses in this region may be involved in the estradiol-induced lordosis behavior, we examined the effect of estrogen on the morphology of synapses in the MCG. Ovariectomized (ovx) adult female rats were given daily subcutaneous injections of estradiol benzoate (10 µg) (m3) or the vehicle control (m3) and after 20 days of injection, only the estrogen-treated (ovx+E) rats showed the lordosis response. MCG tissue was fixed and processed according to standard electron microscopy procedures. A quantitative analysis of MCG tissue demonstrated morphological changes in several synaptic parameters with estrogen treatment. The micrographs were coded and randomly arranged before the analysis, so that the observer was blind to the experimental condition. There was an increase in the mean number of dendro-cored vesicles (dov) per synapse in the MCG of the ovx+E group (1.42±0.04) compared to the ovx group (0.46±0.04) (p<0.0001). The MCG of the ovx+E group also contained a higher percentage (60%) of synapses showing dov+ than the ovx group (28%) (p<0.0001). There was a higher mean length of postsynaptic densities (PSDs) per synapse in the MCG of the ovx+E group (39.7±5.94 nm) than in the controls (15.8±1.7) (p<0.001). In addition, the percentage of PSDs containing one or more perforations was higher (15%) than the controls (10%) (p<0.05). There was also a greater percentage of synapses showing positive curvature in the ovx+E group (82%) compared to the ovx group (62%) (p<0.05). A higher overall number of synapses was seen in the MCG of the ovx+E group (3.6±2.12) compared to the controls (2.7±2.12) (p<0.0001). No significant differences were seen in the percentage of synapses showing subjunctional bodies beneath PSDs in the two groups.

The dov+ are similar in size and appearance to those that increased in number in the VMH of ovx+E animals (Cohen and Pfaff, 1985), suggesting that they may be transported from the zona of uncommitted neurons to a synapse. These dov+ may contain a neurotransmitter, neuromodulator, or a trophic factor (Chung, 1986). Such transmitters or peptides may induce morphological changes in postsynaptic structures which are related to the effects of estrogen on lordosis. Supported by NIH grants HD 07571 and NS 15860.
Synaptic reorganization in the accessory olfactory pathway of rat whose function had been well known was examined for the first step toward understanding the correlation between lesion-induced synaptic reorganization and recovery of function after injury. Time-course of the loss and reappearance of synapses was investigated in the medial amygdaloid nucleus (MAN) following lesions of the accessory olfactory bulb (AOB). A quantitative electron microscopic analysis of the molecular layer was performed after the AOB lesion. Synaptic density was reduced to less than half of the control density at 4 days after the lesion. Thereafter, synaptic density increased more than 80% of control density at 64 days after lesion. The recovery in number of synapses following the lesion orients the possibility of synaptic reorganization by remaining afferent fibers.

Rearrangement of terminations from the fundus of the olfactory nerve was observed in MAN at 2 months following the lesion using an electron microscopy and degeneration study. At 2 days following a BST lesion, the number of degenerating synapses was 0.7 ± 0.1 per unit area in the molecular layer. At 2 months after AOB lesion, the degenerating synapses completely disappeared from MAN. The BST was then lesioned at 2 months after AOB lesion and the degenerating synapses were counted in MAN at 2 days following the BST lesion. The number of degenerating synapses was 3.3 ± 0.6 per unit area in the molecular layer. The number of these degenerating synapses increased significantly in the molecular layer after AOB lesion.

Rearrangement of intra-amygdaloid connections after the AOB lesion was examined further in MAN by use of PHA-L immunohistochemistry. PHA-L was injected in the postero-medial region of amygdala (posterior medial amygdaloid nucleus and amygdala hippocampal transitional area). The labeling axons were observed only in the cellular part of MAN. After the AOB lesion, the labeling axons were found not only in the cellular part but also in the molecular layer. At 2 months after the lesion, the labeling axons were found not only in the cellular part but also in the molecular layer. All these results show that the synaptic reorganization of axons follows the lesion-induced synaptic reorganization and the functional recovery after injury, the study of changes of the accessory olfactory function after the AOB lesion is necessary.

Vasotocin (VT) immunoreactive cell bodies and fibers were demonstrated by immunocytochemistry in the brain of the canary. Canarins invaginated birds were perfused with 10% formaldehyde followed by a two step fixation method: first a low pH and then a high pH of the fixative. Immunoreactive fibers and varicose terminals (50 um) were processed and incubated with antiserum. For visualization of the antigen-antibody reaction we used the avidin-biotin peroxidase complex. Three dorsal cranial cephalic regions contain VT cells: first, the anterior preoptic nucleus and the periventricular magnocellular nucleus (PVM). The PVM is the equivalent of the paraventricular nucleus in the rat; second, the lateral preoptic area, e.g. the suprachiasmatic nucleus in the rat; third, the basal septal area and the nuclear stria terminalis. Axons leaving the PVM together with axons from cells in the lateral hypothalamus form the hypothalamo-hypophysial tract. The medial posterior preoptic nucleus, e.g. the rat ventromedial nucleus shows dense innervation. Extra- hypothalamic areas receive fibers from the PVM. There is a strong innervation of lateral and medial septum, lateral habenula, the substantia grisea centralis, the locus coeruleus, the raphe nuclei, the nucleus tractus solitarius and other cranial nuclei. Many of these nuclei are thought to be involved in mediation of limbic and autonomic function. VT fibers are present in structures related to sensory functions; e.g. the optic tectum and the nucleus ovoidalis. Finally, VT fibers innervate centers which are implicated in vocal control; nucleus robustus archistriatalis and nucleus intercollicularis. Immunoreactive cells and fibers displayed plasticity changes. The male canary shows a denser VT innervation of the lateral septum and has more VT positive fibers in the dorsal diagonal band than the female canary. Castration of male canaries resulted in a decrease of VT immunoreactivity. Chronic testosterone treatment or replacement of the castrated males via silastic implants restored the intensity of the original VT immunoreactive fibers and cells. In the female canary testosterone also increased fiber and cell immunostaining in these brain regions.


Sarventon N-acetylcarnosine transference (NAT) in the rat pineal gland exhibits a circadian rhythm, with activity being highest at night. This rhythm is believed to result from a neurally-induced increase in sympathetic neurons that innervate the gland. NAT activity is abolished by bilateral section of the paraganglionic cervical sympathetic trunks (CS7) or the postganglionic internal carotid nerves (ICN). During the first night after bilateral section of the CS7/CST or the ICN, NAT activity is reduced by 75%. By the second night after such an ICN lesion, NAT activity recovers to normal, but after bilateral section of the CST or the ICN, NAT activity is reduced by 75%. This shows that the lack of recovery in the latter group is due to decentralized sympathetic neurons, which, though electrophysiologically silent, are capable of inhibiting the synaptic efficacy of the contralateral, intact sympathetic nerve. The results obtained by taking up transmitter released by the latter ("heteroneuronal uptake"). When pineal glands are fixed with mixed aldehydes, most varicosities contain small synaptic vesicles with dense cores during the day but not at night, suggesting that the dense cored vesicles are associated with neurons with low firing rates. The NAT-positive nerve endings in animals in which one or both CST were cut, 14%, 52% and 85%, respectively, of the varicosities examined at night were "tagged" with dense cored vesicles. These values correspond well to the expected percentages of innervating neurons which are electrophysiologically silent in the animals. We have used this morphological marker to study the differentiation of "active" and "inactive" varicosities. Varicosities occurred in clusters, and, in animals with one CST cut, these clusters often contained both types of varicosities. In the dorsal raphe, the average distance between an untagged varicosity and the nearest tagged varicosity was 2.2 um., while the distance between an untagged varicosity and the nearest pinealocyte was 2.5 um. These data suggest that varicosities are released from two superior cervical ganglia in close proximity in the pineal gland, and thus support our hypothesis that heteroneuronal uptake may regulate synaptic efficacy. (0112791)


Bilateral adrenalectomy (ADX) produces an increase in brain weight and volume that is arrested but not reversed by later corticosterone (Cort) replacement (1). In young rats this growth takes the form of increased cell division including synaptogenesis and possibly glial proliferation (2). The growth effects which the hormone conditions were changed for half the animals, previously been shown to maintain normal brain size in ADX rats (2). The other animals were given a 2 mg/kg/24 hr dose that was supported by NIH-BMRSG (3). Thus, much is known about the structural changes in the cortex due to ADX and to the hormone conditions. The results revealed a strong association between brain size and cortical thickness, as shown by the cortex of female ADX rats producing maximal long-term potentiation (LTP) at Schaffer/commissural synapses on CA1 neurons in hippocampal slices when the paired bursts were spaced 200 ms apart. A single burst to one set of fibers does not produce LTP but "primes" the post-synaptic neurons such that the de polarization produced by a burst to a second input 200 ms later is much larger and LTP is induced. This phenomenon occurs in both intact and ADX rat brains (3). The average distance between an untagged varicosity and the nearest pinealocyte was 2.5 um. These data suggest that varicosities are released from two superior cervical ganglia in close proximity in the pineal gland, and thus support our hypothesis that heteroneuronal uptake may regulate synaptic efficacy. (0112791)


Short bursts of high frequency stimulation (6 pulses, 100 Hz) produce maximal long-term potentiation (LTP) at Schaffer/commissural synapses on CA1 neurons in hippocampal slices when the paired bursts are spaced 200 ms apart. A single burst to one set of fibers does not produce LTP but "primes" the post-synaptic neurons such that the depolarisation produced by a burst to a second input 200 ms later is much larger and LTP is induced. The present experiments were designed to test the hypothesis that LTP induced by priming is mediated by N-methyl-D-aspartate (NMDA) receptors and that the NMDA component is necessary for the development of synaptic potentiation. The patterned burst stimulation to induce LTP consisted of pairs of bursts to the priming and test inputs separated by 500 ms, the pairs were given ten times at 5 sec. intervals. Dendritic field EPSPs were recorded in response to single-pulse stimulation of the test input, the response to a burst was quantified as the area of negativity evoked by the 4 pulses in the burst. AP5 produced a small depression of the response to the priming burst (0.85±0.33) but a larger and more marked depression of the response enhancement caused by priming (enhancement relative to an unprimed burst was 34.7±9.22 in APS and 32.7±10.1% after washout. NMDA-sensitive AP5 (AP5-1000M) did not significantly alter responses to moderate intensity single pulses. However, it did completely prevent LTP induction by burst stimulation (EPSP potentiation in APS: 0.46±0.81; after washout: 35.7±4.7). APS produced a small depression of the response to the priming burst (0.85±0.33) but a larger and more marked depression of the response enhancement caused by priming (enhancement relative to an unprimed burst was 34.7±9.22 in APS and 32.7±10.1% after washout. NMDA-sensitive AP5 (AP5-1000M) did not significantly alter responses to moderate intensity single pulses. However, it did completely prevent LTP induction by burst stimulation (EPSP potentiation in APS: 0.46±0.81; after washout: 35.7±4.7).

Reactive synaptogenesis has been demonstrated in many regions of the brain in response to a reduction of afferent inputs after environmental insults. Modification in the size, shape, and location of synapses as well as formation of new synapses are important means to restore functional integrity between remaining afferents and their target neurons. Previously, we showed that mature Purkinje cells had a rapid, marked synaptic reorganization following lesioning to granule cells or their parallel fibers. Here we study the reactive changes in the striatum following undernerveing of the frontal cortex in adult rats. The animals were perfused with fixative at 2 to 40 days after surgery. There was a rapid and marked plasticity in the principal caudate neurons. Segregating boutons were numerous but few vacant spines were seen within 2 days after surgery and only a few were still present after 2 weeks. Flagellation of the boutons was prominent 40 days with occasional dense bodies and myelin remnants. Enlarged synapses occurred by 2 days and were evident throughout the period. Dense core vesicles were not found in these enlarged presynaptic boutons indicating that dopaminergic fibers may not be significantly involved in synaptic compensation. There was an accompanying increase in the frequency of multiple synaptic contacts on enlarged boutons. These were from the remaining cerebral cortical connections. The complexity in the shape and size of synapses was analyzed by three-dimensional reconstruction. The enlarged synapses had increased synaptic contact area and a marked irregular boundary with deep indentations. Frequently, perforations occurred as small holes. The contact surface undulated between the spine and bouton generating an enlarged synaptic contact area and intercellular contact zone. The larger synapses had single and double protrusions of the bouton that invaginated the spine head and were capped by synaptic specialization. Quantitative analysis of synaptic profile lengths indicated that the average size of the synaptic area increased at 2 days following lesioning and remained large but was less complex at 40 days. Accompanying this was a decrease in the size of the principal striatal neurons. [Supported by USPH grants NS13942 & HD23494.]

462.12 INCREASED STRIATAL PROTEIN PHOSPHORYLATION IN THE RAT MODEL OF NEUROMUSCULAR PLASTICITY FOLLOWING SUBTOTAL DISUSE. M.A. Fahim, USC Andrus Gerontology Center and Biology Dept., Los Angeles, CA 90089-0191.

The neuromuscular junction (NMJ) undergoes remodeling from early development through senescence but the rapidity with which this occurs and the role of the altered activity have remained unexplored. Muscle activity appears to regulate both presynaptic and postsynaptic activity. In the rat model of disuse (Robbins, N., Trends in Neurosci., 120, 1980; Grinnell, A.D. & Herreras, A.A. Prog. Neuropsychopharmacol., 17, 1981) one of the best documented forms of neurodegenerative disease, which produces about 90% disuse and does not entail use of drugs or nerve injury, is obtained by limb immobilization (Fleisch, G.B. and Robbins, W.J., Physiol. 201, 1969). Since this procedure produces changes in synaptic transmission within 3-5 days, we have examined the short synaptic connections in the motor cortex of immobilized rats for 5 days. Immobilized muscles were then studied both qualitatively and quantitatively. Muscle fiber diameters of immobilized animals were found to be significantly reduced by 30% to 29.5% when compared to those of castrated and control muscles respectively. Evidence of degeneration and regeneration in the same NMJs was observed in all three groups but were significantly greater in the disused muscles. These results reveal, in the adult nervous system, rapid and specific ultrastructural remodeling induced by only a brief period of partial disuse. [Supported by NSF grant BNS-8319639.]


There is growing evidence that vasopressin may serve as a neurotransmitter or neuromodulator in the nervous system and disorders such as kindling. Homozygous Brattleboro rats, kindled (Gillis and Cain, 1983) and repeated intracerebroventricular vasopressin injections have been reported to induce epileptiform activity (Kasting et al. 1980). Kindling (Greenwood et al. 1986). The SHR have been reported to have increased at 3 min. The striata of dyskinetic animals showed significant in­crease in prazosin binding which is accompanied by similar changes in calcium and protein kinase C (PKC)-dependent protein phosphorylation in the brains of IDPN-treated animals (C pe et al. Soc. Neuronol. Abstr. 1986. The present study was carried out to test this idea. Rats were treated with IDPN (100 mg/kg) until the development of the dyskinetic syndrome (7 days). Subsequently, controls and treated animals were killed at various time intervals and their striata were rapidly dissected and frozen in 40%. The buffer made up of 20 mM Hepes, 10 mM MgCl2, 0.5% leupeptin, pH 7.2. Prazosin phosphorylations were started after preincubation with either calcium and/or 0.01% calmidomycin, or phosphorylase b (PB) by the addition of 32P-ATP. The reaction was stopped after 5 min. The striata of dyskinetic animals showed significant in­creases in the PB-dependent phosphorylation of a 66-68 kd and of 100 kd protein. The 66-68 kd protein may correspond to the neurotransmitter protein of similar size which is known to be affected by IDPN treatment. The 100 kd protein may be related to the stress glycoprotein which is induced by calcium channels, glucose deprivation, and stress (Welch et al. J.Biol. 71,1983). The stress glycoprotein is found on both the plasma membrane and the Golgi apparatus. The manner by which IDPN causes these changes and their relation to other biochemical abnormalities re­ported in IDPN-treated animals remain to be determined.

In conclusion we are left with the idea that phosphorylation of neurofilament proteins may play a role in neurodegeneration and in IDPN neurotoxic effects. These data also suggest that studies of phosphoryl in human dyskinetic disorders may be a fruitful area of research.


The neuromuscular junction (NMJ) undergoes remodeling from early development through senescence but the rapidity with which this occurs and the role of the altered activity have remained unexplored. Evidence of degeneration and regeneration in the same NMJs was observed in all three groups. The effect of 5 days immobilization on muscle fiber diameters of immobilized animals was found to be significantly reduced by 30% to 29.5% when compared to those of castrated and control muscles respectively. Muscle activity appears to regulate both pre- and postsynaptic activity. In the rat model of disuse (Robbins, N., Trends in Neurosci., 120, 1980; Grinnell, A.D. & Herreras, A.A. Prog. Neuropsychopharmacol., 17, 1981) one of the best documented forms of neurodegenerative disease, which produces about 90% disuse and does not entail use of drugs or nerve injury, is obtained by limb immobilization (Fleisch, G.B. and Robbins, W.J., Physiol. 201, 1969). Since this procedure produces changes in synaptic transmission within 3-5 days, we have examined the short synaptic connections in the motor cortex of immobilized rats for 5 days. Immobilized muscles were then studied both qualitatively and quantitatively. Muscle fiber diameters of immobilized animals were found to be significantly reduced by 30% to 29.5% when compared to those of castrated and control muscles respectively. Evidence of degeneration and regeneration in the same NMJs was observed in all three groups but were significantly greater in the disused muscles. These results reveal, in the adult nervous system, rapid and specific ultrastructural remodeling induced by only a brief period of partial disuse. [Supported by NSF grant BNS-8319639.]
462.15 CONTRIBUTION OF INHIBITION TO THE COOPERATIVITY THRESHOLD FOR LONG-TERM POTENTIATION.

R.M. Douglas and A. Dempster, Dept. Physiology and Biophysics, Dalhousie University, Halifax, N.S., Canada.

Long-term potentiation (LTP) of perforant path synapses is only induced when a relatively large number of the excitatory synapses are active (McNaughton et al., Brain Res., 1976). The cooperativity requirement for LTP can be met by using moderate stimulation intensities to activate a high percentage of perforant path axons. In the presence of GABA antagonists, LTP is blocked by activation of an inhibitory pathway to the dentate area (Douglas et al., Brain Res., 1982), and addition of bicuculline, a GABA antagonist, greatly enhances the amount of LTP observed in vivo (Wigstrom & Gustafsson, Nature, 1983).

Characteristics of these neurons are summarized in the table below.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Normal Nerve Cut</th>
<th>With IO Receptive Fields</th>
</tr>
</thead>
<tbody>
<tr>
<td>No field</td>
<td>62.1%</td>
<td>91.4%</td>
</tr>
<tr>
<td>Interdivisional convergence (excluding whisker convergence in TH cells)</td>
<td>1.9%</td>
<td>2.1%</td>
</tr>
<tr>
<td>Cervical convergence</td>
<td>1.3%</td>
<td>2.3%</td>
</tr>
<tr>
<td>Celloi with IO receptive fields</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whisker responsive, light touch</td>
<td>63.6%</td>
<td>87.4%</td>
</tr>
<tr>
<td>Guard hair responsive, light touch</td>
<td>21.7%</td>
<td>5.7%</td>
</tr>
<tr>
<td>Skin responsive, light touch</td>
<td>8.4%</td>
<td>6.9%</td>
</tr>
<tr>
<td>Pinch or deep pressure</td>
<td>10.8%</td>
<td>6.9%</td>
</tr>
<tr>
<td>Split receptive fields</td>
<td>0.0%</td>
<td>2.7%</td>
</tr>
<tr>
<td>Spontaneously active</td>
<td>28.9%</td>
<td>18.4%</td>
</tr>
<tr>
<td>Celloi with mystacial whisker fields</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guard hair or skin convergence</td>
<td>28.3%</td>
<td>3.9%</td>
</tr>
<tr>
<td>LC neurons responding to LC whisker</td>
<td>3.7%</td>
<td>5.2%</td>
</tr>
</tbody>
</table>

Thus, chronic effects of adult IO nerve transection include changes in 1) numbers of SpVi cells activated from the IO region of the face, 2) receptor modalties driving SpVi cells, 3) intra- and inter-receptor modality convergence, 4) inter-divisonal convergence, 5) cervical convergence and 3) receptive field continuity (sp. split fields). The respective roles of primary and central afferents to SpVi in the functional alterations noted above, await further clarification. Support DE07734, DE07662, A0 A.

462.16 THE CAPACITY FOR NEUROGENESIS AND AXON RECONNECTION PERSISTS IN THE OLD ADULT NERVOUS SYSTEM.

R.S. Contantos and R.E. Morrison, Dept. of Physiology, Medical College of Virginia, Richmond, Va.

It has been shown that neurogenesis, cell replacement and axon reconstruction occurs in the olfactory system of young animals. In addition, newly developing axons have been shown to reestablish functional connections with cortical cells in the olfactory bulb (Br. J. Exp. 361:258-266, 1983).

In the present study we examined the capacity of the newly developed axons to reestablish contact with the mature brain (olfactory bulb) in old adult hamsters. A new method was developed for making complete transection of sensory fibers projecting to the olfactory bulb. Non-invasive techniques were used to examine the extent of developing axon projections originating from olfactory receptor cells. We observed axon projections growing from receptor neurons across the transection site to the olfactory bulb. Further examination of the olfactory bulb revealed that axon terminals formed glomerular-like structures and made synaptic connections with second order cells in the olfactory bulb. The results demonstrate that the mammalian central nervous system of old adult animals maintains the capacity for neurogenesis and axon reconstruction. These findings may have important implications for replacement and repair of damaged pathways in the adult CNS. Supported by MHS Grant NS16741 to RMG.
462.19 NEUROPEPTIDE-Y REGULATES CAPSAICIN-INDUCED CALCIUM UPTAKE INTO CULTURED DORSAL ROOT GANGLION (DRG) NEURONS. J. Winter* and G. Ryan* (SPIN: F. Kirkwood). Sandusky Institute for Medical Research, 5 Gower Place, London WC1E 6BN.

Capsaicin acts selectively on a subpopulation of rat DRG neurons, namely those involved in nociception. In vivo capsaicin application causes excitation of these neurons resulting in a painful burning sensation; capsaicin-induced excitation in vitro results in accumulation of high concentrations of calcium (measured using 45Ca2+) into primary cultures of neonatal rat DRG neurons but not sympathetic neurons or non-neuronal cells.

Whereas neonatal DRG neurons require NGF for survival, adult DRG neurons need no growth factors and can be used to study neuro-modulatory effects of NGF (e.g. neuropeptide levels, see Lindsay, Medical Research, 5 Gower Place, London WC1E 6BN.

In response to capsaicin, adult rat DRG neurons grown in the presence of ligand of NGF took up 4-6 times the amount of Ca2+ taken up by neurons grown in the absence of NGF. Approximately 60 p mol of Ca2+ were taken up per 1000 NGF-treated neurons during a 10 minute incubation with capsaicin. The amount of Ca2+ uptake was dependent on the concentration of NGF and was maximal at 200 ng/ml. Removal of NGF caused a reduction in responsiveness to capsaicin. The reduced Ca2+ uptake in the absence of NGF is not due to loss of capsaicin-sensitive neurons; when NGF is omitted from these cultures for 6 days then added back for 6 days, the level of Ca2+ uptake is the same as for 6 day old neurons grown in the continuous presence of NGF. In parallel cultures, the number of neurons that could be killed by capsaicin increased with time in culture, from c.15% on the second day, to 30% at day 7.

These results suggest that NGF can regulate DRG neuron responses to capsaicin, possibly by modulation of capsaicin receptors and/or calcium channels on the surface of these cells.


Previous research (Boyesen & Feeney Neurosci. Abst., 1984) has indicated that a depression in noradrenaline (NE) function occurring in the cerebellum contralateral to a sensorimotor cortex (SMC) injury in rats. The resolution of the NE depression, thought to be regulated by the Locus Ceruleus, may be induced by infusions of NE into the contralateral cerebellum. The NE infusion results in a remarkable recovery from the hemiparesis induced by the SMC injury. The present experiment suggests that the depression in NE function in contralateral cerebellum is a result of damage to terminal LC fibers in SMCX which simultaneously innerserves cerebellum. If the hemiparesis following SMCX injury is, in part, mediated by a transient depression in NE function in cerebellum induced by damage to the cortical NE projection, then predepletion of cortical NE should serve a protective effect on hemiparesis following SMCX injury. Our results indicate that dorsal bundle lesions followed in 2 weeks by a unilateral SMCX injury results in a very small amount of hemiparesis. Dorsal bundle lesions alone were found to have no effect on hemiparesis as measured by the beam walking task. These results are consistent with the hypothesis that a remote functional depression occurs in the cerebellum contralateral to the SMCX injury that is mediated by the locus ceruleus. In support of this hypothesis is the finding that localized micoinfusions of phenoxylbenzamine, an alpha receptor antagonist, into the contralateral cerebellum reinstated the hemiparesis in animals long since recovered from the SMCX injury. Supported by NIH 1R23-NS23003 to MGB.
EVIDENCE THAT LHRR AND THE LHRR FRAGMENT, AC-LHRR5-10, FACILITATE MATING BEHAVIOR IN THE FEMALE RAT VIA DIFFERENT MECHANISMS. C.A. Dudley and R.L. Moss. Dept. of Physiology, University of Texas Health Science Center, Dallas, TX 75235.

Both LHRR and the Ac-LHRR5-10 fragment have been demonstrated to facilitate mating behavior in the ovariectomized, estrogen-primed female rat when infused intraventricularly or into specific CNS sites known to be involved in mating behavior. However, the Ac-LHRR5-10 fragment does not possess LH-releasing activity, either in vitro or in the intact rat. The physiological actions in which both Ac-LHRR5-10 and LHRR were applied to neurons in the ventromedial nucleus indicate that the two peptides are capable of exerting different effects on firing rate of the same neuron. These findings suggest that the two peptides may achieve their behavioral actions by different mechanisms. This possibility was examined by determining if a potent LHRR antagonist analog produced differential blockade of Ac-LHRR and Eα-Ac-LHRR8-10 induced sexual behavior in the female rat.

Sprague Dawley female rats weighing 220-240 g at the time of arrival were housed in individual cages in a temperature-controlled room with a reversed light-dark cycle (lights off from 1100h to 2100 h). The animals were bilaterally ovariohysterectomized under ether anesthesia and, two weeks later, were anesthetized with Equithesin and placed in a stereotaxic instrument for bilateral implantation of 23 gauge stainless steel cannulas directed towards the VMH. A preliminary mating test was conducted two weeks after surgery. All animals were primed with luteinizing hormone-releasing hormone (LH-RH, 100 ng) and infused under ether anesthesia with either the LH-RH antagonist, AcProl, GLC D-Phe2, D-Tyr3,6 (100 ng) or with saline. Mating behavior tests were conducted at 48 h and consisted of placing the female rat in a semicircular plexiglass mating arena containing two sexually active male rats. The presence or absence of lordotic posture as well as the degree of lordotic arching in response to 15 male copulatory contacts or for 20 min was noted. These were presented in terms of the lordose to mount ratio (L/M, number of lordoses/number of male contacts). To be included in the study, the animals had to show an L/M of 61 or greater for the E-P test. Two weeks later, the animals were primed with LH-RH at 0 h and infused under ether anesthesia with either the LH-RH antagonist, AcProl, GLC D-Phe2, D-Tyr3,6 (100 ng) or saline at 48 h. At 46.75 h, the animals were infused with either LH-RH or Ac-LHRR5-10 (100ng) and mating behavior tests were conducted at 48 h. Estrone-LH-RH5-10 (100 ng) and estradiol-LH-RH5-10 (100 ng) were used as controls.

Estrone-LH-RH5-10 (mean L/M: 71; n=12) was significantly increased (p < 0.05) and estradiol-LH-RH5-10 (mean L/M: 71; n=12) was not affected by infusion of the LH-RH antagonist (mean L/M: 71; n=12). Thus, competitively blocking LH-RH receptors with the ability of Ac-LHRR5-10 to enhance mating behavior. These results support the hypothesis that the LHRR fragment and the LHRR decapasse induce differential mating behavior via different mechanisms. Supported by NIH grant MH41784.

PRESSOR RESPONSES TO ANGIOTENSIN, BESTATIN AND ALL ARE SUPPRESSED BY PRETREATMENT WITH ANGIOTENSIN RECEPTOR ANTAGONIST SARTRAN. C.M. Batt, J.L. Wolf, C.J. Sweitzer, and H.E. Klein*. Departments of Psychology and Speech, University of Washington, Seattle, Washington 98195.

The octapeptide angiotensin II (AI) has generally been considered the active peptide of the central renin angiotensin system. However, recent reports have found intracerebroventricularly (icv) applied angiotensin III (III, heptapeptide) to be equipotent with AI as a pressor agent (Fink et al., 1985; Wolf et al., 1985). Utilization of microwaves to fix peptidase activity in conjunction with HPLC analysis has also recently shown that centrally injected iodinated AI has a half-life of 23 sec as compared with 8 sec for AII (Harding et al., 1986). An analysis of structure-activity relationship and signal termination at the angiotensinergic receptor at which LH-RH is reported to act raises the possibility that LH-RH receptor antagonists may be useful in the treatment of hypertensive states. The present study investigated the effects of sarthran (Sar,Thr-AII), a specific angiotensin receptor antagonist, on the pressor responses of AI, BE, and All. 

Sarthran (10 nmoles in 2 ul aCSF), All (10 or 100 nmoles in 2 ul aCSF), or AI (10 or 100 nmoles in 2 ul aCSF) were given at 50, 60 and 70 sec after sarthran. The results indicate that at the 90-minute timepoint. Since sarthran is a specific angiotensin receptor antagonist, it is likely that the increased pressor responses observed are due to the action of the endogenous central angiotensinergic system rather than some other peptide system.

The results indicate that AI, BE, and All are having their pressor effects via the central angiotensinergic system. The patterns of AI, BE and All recovery from the influence of a specific angiotensin receptor antagonist are very similar.

INCORPORATION OF RELEASING FACTOR (CRF) RECEPTORS IN THE NEONATAL RAT SPINAL CORD: EVIDENCE FROM AUTORADIOGRAPHIC AND ELECTROPHYSIOLOGICAL STUDIES. J.A. Bell* and E.B. de Souza (SPON: G. Battaglia). Neuroscience Branch, NIH AFRIGENESIS RESEARCH Center, Baltimore, MD 21224.

Accumulating evidence suggests that CRF may play a role as a neurotransmitter in the CNS to coordinate the organism's endocrine, behavioral and autonomic responses to stress. The autoradiographic study of the present investigation was to determine if CRF binds specifically to the plasma membrane of specific cell types in the hypothalamus. The results further suggest that the increased half-life of AII via icv BE treatment is responsible as compared with blood pressure elevations due to AI all or BE alone. Since BE is known to increase blood pressure in rats, it is possible that BE may be acting on the central angiotensin system rather than some other peptide system.

The results indicate that AI, BE, and All are having their pressor effects via the central angiotensinergic system. The patterns of AI, BE and All recovery from the influence of a specific angiotensin receptor antagonist are very similar.
463.5 SINGLE-PULSE STIMULATION OF THE MEDIAL EXFERMENT PATHWAY WITH CONCURRENT ADMINISTRATION OF CCK 8-S APPEARS TO POTENTIATE THE RAT SATE SATIE ORTH ESP. (DOUBLE-FAH, Research Department, CIBA-GEIGY Corp., Summit, NJ 07901 and The Rockefeller University, NY, NY 10021.

Long-term Potentiation (LTP) is defined as a use-induced increase of the population EPSP and then remains stable for an extended period of time. It is a form of synaptic plasticity that is thought to underlie learning and memory. In this study, the authors investigated the role of CCK 8-S in regulating LTP.

It has been previously reported (Dahlgren and Winblad, Fed. Proc., 46, 3, 1987) that cholecystokinin (CCK), which is a potent neurotransmitter, can influence LTP. In this study, the authors administered CCK 8-S to rats and observed the effects on LTP.

The results showed that CCK 8-S increased the amplitude of the EPSP and prolonged the duration of LTP. These findings suggest a potential role for CCK 8-S in modulating synaptic plasticity and learning processes.

463.6 SECRETIN FAMILY PEPTIDES ENHANCE SYNAPTIC TRANSMISSION IN RAT SYMPATHETIC GANGLIA. D.G. MacKenna*, D.A. McAffee, C.A. Briggs; Beckman Res. Inst. Duarte, CA, Abbott Labs, Chicago, IL, Nelson Research, Irvine, CA, 92715.

Neuropeptides appear to play a role in a number of autonomic functions. These substances raise cyclic 3',5'-adenosine monophosphate (cAMP) levels in various tissues including autonomic ganglia (Ip, N.Y. et al, J. Neurosci. Res., 13, 1984). Substances such as secretin, secretin-like peptides, and CCK 8-S, are known to activate adenylate cyclase, which in turn, leads to an increase in intracellular cAMP levels.

In this study, the authors investigated the effects of secretin and secretin-like peptides on synaptic transmission in rat sympathetic ganglia. They found that these peptides enhanced synaptic transmission by increasing cAMP levels, which in turn, potentiated the release of neurotransmitters.


Insulin interacts with glucoregulatory mechanisms in the central nervous system (CNS) where it affects energy metabolism in a number of ways. These include the regulation of glucose uptake, glucose metabolism in the brain, and the regulation of glucose homeostasis.

The authors investigated the mechanism by which insulin affects glucose metabolism in the brain. They found that insulin induces an increase in glucose uptake and its subsequent metabolism in muscle and adipose tissues. The results suggest that insulin-induced glucose uptake is mediated by a phospholipid-dependent mechanism.


Angiotensin II and peptide Y (PYY) are potent neuropeptidic agents acting at several circumventricular regions in the brain. These peptides also constrict cerebral vessels in vitro or in situ and therefore may influence blood flow in the circumventricular organs. The effect of these peptides on blood flow was measured.

The results showed that angiotensin II increased local cerebral blood flow, whereas peptide Y decreased it. These findings suggest a potential role for these peptides in regulating local cerebral blood flow and in the regulation of blood flow in other regions of the brain.


Local cerebral blood flow was measured 15 minutes following the infusion of angiotensin II and peptide Y. The results showed that angiotensin II increased local cerebral blood flow, whereas peptide Y decreased it. These findings suggest a potential role for these peptides in regulating local cerebral blood flow and in the regulation of blood flow in other regions of the brain.


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463.9 POTENTIATION OF BRADYKININ-INDUCED NOCEPTION BY ANGIOTENSIN CONVERTING ENZYME INHIBITORS. R.G. Browne. Research Department, Pharmaceuticals Division, Ciba-Geigy Corp., Summit, NJ 07901

Bradykinin is an endogenous agrinergic mediator which elicits pain upon intradermal injection in man. In guinea pigs, the close-arterial injection of sub-microgram quantities of bradykinin elicits a brief vocalization response which can be blocked by the prior administration of various analgesic drugs (Ascoli and Borne, Pharmacol. Rev. 20: 117-124, 1968). Bradykinin is rapidly metabolized by at least two kinases, and one of these enzymes, known to be inhibited by the angiotensin converting enzyme (ACE) inhibitor captopril (Regoli and Barabe, Pharmac. Rev. 32: 1-46, 1980). In fact, some side effects of ACE inhibitors have been attributed to increased bradykinin actions (Sesko and Maroko, Arch. Int. Pharmacod. 145: 1224, 1986). The present experiment was designed to test the hypothesis that kinase II inhibition by captopril would result in a potentiation of the nociceptive effects produced by intra-arterial bradykinin injection.

Male Duncan Hartley guinea pigs (ELM:DH) weighing 300-350 g were maintained in single cages under standard laboratory conditions. Medical grade polyvinyl catheters (0.20 mm id x 0.50 mm o.d., Dural Plastic) were implanted, under ketamine (Vetalan, Parke-Davis) anesthesia, into the right femoral artery. At least 48 hrs after surgery the guinea pigs were administered varying concentrations of bradykinin (Bachem) in a volume of 0.1 ml followed by flushing the catheter with 0.3 ml of saline. The animals were individually observed for the presence of a brief (less than 60 sec duration) vocalization response characterized by at least three clearly audible "squeals" over the two min period following the injection. For experiments involving bradykinin interactions 200 ug of either captopril (Squibb) or enalapril (Merck) were injected into the arteries of a volume of 0.1 ml 30 min before re-testing for the presence of vocalization with bradykinin.

The results of this study demonstrate a marked potentiation of bradykinin's nociceptive actions by the ACE inhibitors captopril and enalapril. In guinea pigs, vocalization was observed at sub-microgram doses (threshold doses for individual animals ranged from 0.25 to 0.25 pg). Captopril pretreatment shifted the dose response for bradykinin-elicited vocalization toward the left. In fact, following captopril, greater than half the animals were observed to emit a vocalization response at previously sub-threshold concentrations of bradykinin. These results support the role of kinase II in the degradation of bradykinin and are the first to demonstrate a potentiation of bradykinin-induced nociception by ACE inhibitors.

463.10 LONG TERM CHANGES IN SYNAPTIC ACTIVITY INDUCED BY THE MAST CELL DEGRANULATING PEPTIDE IN CA1 AND CA3 HIPPOCAMPAL NEURONES

E. Cherubini, R. Neuman, M. Gho and Y. Ben-Ari. INSERM U 275, 123 Bd. du Port Royal, 75475 Paris France.

The mast cell degranulating peptide (MCD) which has been isolated from bee venom, induces these rhytm in the hippocampus and paraventricular area upon central administration in rats (Bider et al., Brain Res., 1987, in press). These effects are mediated by high affinity binding sites concentrated in cortical structures, notably in the hippocampus (Ibid). The present experiments were performed to study the mechanism of action of MCD. In rat hippocampal slices, a brief application (2-5 min) of MCD (0.1-2.0 µM) induced in CA1 a long lasting enhancement (> 3 hours) of the field and intracellular app activity produced by Schaffer collateral stimulation. At the different levels, the post synaptic cell excitability was not changed by MCD. The action of MCD is therefore reminiscent of the long term potentiation (LTP) produced by a train of high frequency electrical stimulation (Cherubini et al., Nature, 1987, in press). In the CA3 region, MCD (0.5-2 µM) produced spontaneous bursts that persisted for several hours. These bursts are activity driven (not endogenous pacemaker burst) and the underlying paroxysmal depolarizing shift is a giant evoked spike with a reversal potential close to 0 mV. Furthermore, following application of MCD, stimulation of the mossy fiber or commissural pathways evoked a conventional app followed by an evoked network burst (ENB). The ENB which could be induced for several hours, was not blocked by bicuculline or elevated divalent cation concentrations. Concomittantly, but not subsequent applications of 7X (1 µg) or cobalt (1 µM) blocked the long lasting effects suggesting that the action of MCD requires synaptic activity.

Therefore, MCD produces long lasting changes in the synaptic response both in CA1 and CA3 region. It is possible that acting presynaptically, MCD releases protein/e and/or amino acids which will act postsynaptically to produce the long lasting changes. Since an endogenous ligand for MCD has been found in brain extracts with receptor and radio immuno assays (Cherubini et al., Nature, 1987, in press) the present results suggest that a MCD like agent may be involved in the long lasting changes in synaptic activity.

M. Gho is a fellow of Fondation Fyssen.


We synthesized two peptides encoded by a hypothetic mRNA sequence, complementary to the mRNA for rat growth hormone releasing hormone (GHRH). The peptide was produced by the complementary mRNA in the 3'-5' direction (3'-5'CP) and is complementary to the amino acid residues 14-25 of the two CPs match the hydropathic pattern of GHRH (22-27). Cyclic nucleotide mediation for FMRFamide response was suggested because the FMRFamide response was enhanced by perfusion with phosphodiesterases inhibitors, IBMX (1 µM) or theophylline (1 µM). Possible second messengers of FMRFamide were investigated. Cyclic AMP (cAMP) and cyclic GMP (cGMP) were injected into the neuron and the response recorded by means of a two-electrode voltage clamp system. A cAMP-induced current that was similar to the response of Na to FMRFamide has been reported in other molluscan neurons (Conner and Hochberger, J. Physiol. 354: 139-162, 1984). However, cAMP (100 µM, several pl) injected into R14 induced a dose-dependent and sustained outward current (up to 10 nA, almost 30 min). When cGMP (50 µM, several pl) was injected into the neuron, an inward current similar to the FMRFamide response was observed. The cGMP induced inward current increased with depolarizing holding potentials from -70 mV, maximally at about -20 mV. Partial replacement of sodium in the recording solution did not change the cGMP response. Replacing external chloride with gluconate increased the cGMP induced inward current, sodium free solution eliminated the inward current. Changing the external potassium concentration did not change the cGMP response. Replacing external chloride with gluconate increased the cGMP induced inward current, sodium free solution eliminated the inward current. The cGMP induced inward current and on the FMRFamide induced inward current were similar. Furthermore, when a few drops of the guanylate cyclase activator 8-bromo cyclic GMP (150 µM) was applied to the bath an inward current similar to the FMRFamide response was observed. These results suggest that cGMP mediates the FMRFamide response in R14.

First introduced for the treatment of migraine and cluster headaches more than 50 yrs ago, the ergot alkaloids remain the most effective drug therapy for headaches. Although the ergots are presumed to work by constricting painful, dilated cephalic vessels, evidence is lacking to suggest that either or any vasocostrictor mechanism of drug action. In this report we provide evidence that plasma extracellular (PE) which occurs following depolarization of the trigeminal (V) nerve is blocked by ergot alkaloids that are C-fiber dependent.

Our first studies demonstrated that PE developed within the dura following capsaicin treatment as neonates. Moreover, acute pretreatment with capsaicin (i.v.) or electrical V stimulation in adult rats resulted in altered PE responses. In the absence of unmyelinated C-fibers in as much as PE was not presumed to work by constricting painful, dilated cephalic vessels, the development of enhanced permeability was dependent upon the presence of unmyelinated C-fibers in as much as PE was not measurable in adult animals in whom C-fibers were depleted by capsaicin treatment as neonates. Moreover, acute pretreatment of animals with 11-branched neuropeptides (NOS), enkephalins, neurotransmitters (TNT) or chronic methylprednisolone blocked the capsaicin response in doses similar to those used clinically. All three drugs were also effective against electrical stimulation, although at higher doses.

To support a role for the neuropeptides as mediators of neurogenic PE in the dura mater, and to evaluate the role of vasoconstriction as a possible mechanism of ergot action, the ability of sensory peptides to induce PE was investigated. SP, NKA and capsaicin were effective, an effect that was C-fiber independent and largely the result of a direct vascular action. Neither BST or BST pretreatment blocked the SP or NKA-induced response even when administered in amounts much greater than those required to block capsaicin-induced PE. This observation combined with the demonstration that two potent vasoconstrictors angiotensin II or endothelin did not block capsaicin-induced PE suggests that vasoconstriction is not the major mechanism by which these neuropeptides promote PE and raises the possibility that ergot compounds act directly to alter the ability of the sensory nerves to promote PE, perhaps by interfering with the release of mediators such as histamine as has been suggested in the case of opiate peptides.


Intracisternal i(c) injections of bombsin in rats causes hyperglycemia, suppression of gastric acid secretion (Taché, et al., Proc. Natl. Acad. Sci. USA 77:1515, 1980), and the attenuation of stomach erosions induced by cold-stress restraint (Taché, et al., Life Sci. 29:91719, 1979). Both effects are mediated by a bombesin-like peptide that enhances hyperglycemia and suppresses gastric secretion when administered to the brain (Taché, et al., Science 222:935, 1983). The present study was conducted to examine whether either or both of these effects prevent the formation of stomach erosions induced by lateral hypothalamic lesions or rats and the effect of CRF administration and LH lesions on gastric acid secretion, serum gastrin and glucose levels.

In one experimental group of 9 male rats were matched for body weight. All groups were food deprived overnight, anesthetized with sodium pentobarbital, and then 4 groups were given bilateral electrolytic LH lesions. The fifth group was given sham surgery. Immediately following surgery the LH groups were injected (i.c.) with either bombsin (900 ng) or saline vehicle (10 μl). All groups were food and water deprived for 24 h post-operatively, sacrificed by decapitation, and the stomachs examined. Mean total area (cm²) of gastric damage was as follows: Sham-Veh (15.15), LH-Veh (23.2), LH-CRF (15.7), LH-tCRF (14.8), LH-Bomb (6.1).

In the second experiment, groups of rats that were sham operated or given LE, were also given the same CRF, or saline vehicle, and then gastric secretions were collected. Group mean values are presented in the table below:

<table>
<thead>
<tr>
<th>Group</th>
<th>Volez</th>
<th>pH</th>
<th>Acid Output</th>
<th>Gastrin</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-Veh</td>
<td>2.63</td>
<td>1.4</td>
<td>126</td>
<td>100</td>
<td>190.3</td>
</tr>
<tr>
<td>Sham-CRF</td>
<td>0.88</td>
<td>4.1</td>
<td>27.2</td>
<td>100</td>
<td>190.3</td>
</tr>
<tr>
<td>LH-Veh</td>
<td>3.04</td>
<td>1.3</td>
<td>326.9</td>
<td>76</td>
<td>123.3</td>
</tr>
<tr>
<td>LH-CRF</td>
<td>0.93</td>
<td>1.7</td>
<td>126.9</td>
<td>76</td>
<td>123.3</td>
</tr>
</tbody>
</table>

The results show that bombesin attenuated the formation of gastric erosions after LH lesions (p<0.05), whereas CRF did not. However, CRF effectively suppressed gastric acid output in LH lesions (p<0.01). These results suggest that bombesin and CRF may act through different mechanisms to reduce stomach erosions by different mechanisms. The results also show that LH lesions prevented the hyperglycemic effects of CRF, reinforcing the idea that the LH area is a primary site for glucostatic effects (NIH Grant NSO60, USPHS Biomed. Research Support Grant RR7099).

463.15 REGULATORY PEPTIDE CONCENTRATIONS IN HUMAN SYNOVIAL FLUID OBTAINED FROM ARTHRITIC KNEE JOINTS. P. Roddy*, W. Ginsburg*², J. Bailey*, R. Valente*², V.L.W. Go*¹, D. Lucas*¹, A. Morris*¹, W. Reilly*², R. Graph*², TLY.*¹

Obtained from inflammatory and non-inflammatory conditions.

DJD (n=5) 377 31 41 490 28 295 106 59

Conclusions: The relevance of increased BLI, SP and G to the pathophysiology of RA remains to be determined. The regulatory peptides in peripheral tissues. Organic dysfunction may be mirrored in changes in the circulating levels of these agents. The present studies seek to concurrently establish baseline profiles of these hormones in the plasma of normal males and females, and to determine the relationship of these levels to age, sex, and menopausal status.


Current evidence suggests that the presence of a variety of neuropeptides in peripheral tissues may modulate afferent neural activity and proliferation of lymphocytes and synoviocytes. To determine whether differences in synovial fluid concentrations of neuropeptides exist in arthritic and non-arthritic patients, the concentrations of these agents were measured in plasma obtained: (1) from patients with rheumatoid arthritis (RA); (2) polyneuropathy (PM); (3) Pseudohyphal (PS); (4) idiopathic (ID); and (5) non-inflammatory (degenerative joint disease: DJD). Plasma samples were obtained from patients with arthritis diagnoses: RA, polymyalgia rheumatica (PM), PNS, PNS, ID, and DJD.

The results show that bombesin attenuated the formation of gastric erosions after LH lesions (p<0.05), whereas CRF did not. However, CRF effectively suppressed gastric acid output in LH lesions (p<0.01). These results suggest that bombesin and CRF may act through different mechanisms to reduce stomach erosions by different mechanisms. The results also show that LH lesions prevented the hyperglycemic effects of CRF, reinforcing the idea that the LH area is a primary site for glucostatic effects (NIH Grant NSO60, USPHS Biomed. Research Support Grant RR7099).

Results:

<table>
<thead>
<tr>
<th>zahl</th>
<th>Concentration (pg/ml)</th>
<th>SEM</th>
<th>Mean</th>
<th>SEM</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA (n=5)</td>
<td>12,057</td>
<td>128</td>
<td>580</td>
<td>72</td>
<td>128</td>
</tr>
<tr>
<td>PM, P, I</td>
<td>18,261</td>
<td>43</td>
<td>83</td>
<td>46</td>
<td>101</td>
</tr>
<tr>
<td>DJD (n=5)</td>
<td>377</td>
<td>31</td>
<td>41</td>
<td>298</td>
<td>295</td>
</tr>
<tr>
<td>Other</td>
<td>4,289</td>
<td>20</td>
<td>215</td>
<td>28</td>
<td>184</td>
</tr>
</tbody>
</table>

p<0.01 NS N | <0.05 NS N | <0.05 NS N |

One-way analysis of variance among the 3 groups.

In RA, concentrations of BLI, SP and G appeared increased compared to ODD and other non-RA inflammatory disorders, and HPP was decreased compared to ODD. In non-RA inflammatory disorders, CCK, G, and PG were increased and HPP was decreased compared to DJD. Conclusion: The relevance of increased BLI, SP and G to the pathophysiology of RA remains to be determined. The regulatory peptides in synovial fluid may be released locally by synoviocytes and/or mast cells and, therefore, may influence the permeability of the synovial membrane. This remains to be elucidated. (Supported by NIH grant AI-34988, TLY.)

Substance P (SP) is contained in the peripheral terminals of small primary afferents. In the present studies we sought to determine whether these peripheral stores could be released into the extracellular space surrounding the nerve terminal. We accomplished these experiments, halothane (0.6%) analgesized, artificially ventilated and artificially perfused animals were used. Sciatic nerve electrical and capacitative nerve electroexsions were placed at the ischial notch and the nerve cut proximally to the spinal cord. To perfuse the extracellular spaces, three approaches were taken: 1) knee joint perfusion (KJ) performed by the instillation of inflow and outflow needles placed into the synovial fluid; 2) intradiscal perfusion of a 0.5% DgKREB on the dorsum of the desipated paw with inflow and outflow needles; or 3) collection of spontaneously draining lymph (LM) following catheterization with PE-10 of the saphenous vein duct just below the sciatic nerve. Perfusions were carried out with a modified Krebs solution at 50 µl/min using a two channel peristaltic pump for each site. Samples were assayed for sP using antibody #4892. This antibody cross-reacts with SP1/4/6 and SP1/4/9 and it shows a progressive loss of cross reactivity following more extensive removal of either N or C terminal amino acids. In unstimulated samples, levels of sP were frequently undetectable in K/SH (1-3 pg/ml). Stimulation of the sciatic nerve (30 V, 1.0 msec, 50 Hz, 1 sec off) resulted in significant reversible increases in sP-like immunoreactivity at all sites. Addition of capsaicin (3 x 10^-6 M) to the perfusate also resulted in significant increases in sP levels. The effects of sciatic nerve stimulation following cap­saicin was diminished. To determine the role of neurotransmitters, the addition of serotonin (10^-5 M) to the perfusate abrogated the evoked release of SP in the KJ and SB perfusates. These data suggest that the peripheral terminals of small primary afferents may possess a pharmacologic characteristic in sP release compared to that of the central terminals. (Supported by NIH grant NS-16541, TLY.)

Levels of sP-like Immunoreactivity in Effluent: x ± SE (pg/ml)

Control 3.20±0.1 (10) 1.92±0.02 (10) 265±5 (5)
Sciatic nerve 47.2±1.5 (6) 31.6±1.6 (6) 492±4 (4)
Capsaicin 59.1±1 (10) 49.2±1 (10)
Sciatic nerve 7.5± (6) 10±8 (6)


Clonidine-displacing substance (CDS) is a low-molecular-weight endogenous substance in brain (Atlas & Burnett, Eur J Biochem 144:287, 1984; Meeley et al., Life Sci 38:1119, 1986). We sought to determine whether CDS, like many neurotransmitters, is biologically active on smooth muscle. CDS was partially purified from bovine brain (Meeley et al., op cit). Smooth muscle preparations were suspended on force transducers with silk suture and superfused with oxygenated Krebs' buffer at 10 mL/min. Bolus applications of CDS had no effect on arterial (rat and rabbit aorta) or venous (dog and rabbit saphenous vein) vascular smooth muscle. The effects of CDS on rabbit jejunum and ileum were biphasic (contraction/relaxation) and variable. In contrast, CDS induced rapid, consistent, and dose-dependent contractions of the rat gastric fundus. In order to characterize the response to CDS, contractions were standardized to the peak tension elicited by prostaglandin E2 (PGE2, 200 pmoles). One Unit of CDS (equivalent to the amount of brain extract required to inhibit 7H-p-aminoclonidine binding by 50% under standard conditions) induced a contraction which was 82 ± 2% of that elicited by PGE2. To determine whether CDS acted directly on fundic smooth muscle or on intrinsic neurons, fundic strips were continuously superfused with tetrodotoxin (TTX, 0.1 µM). The response to CDS was not blocked, but slightly facilitated (peak tension = 113 ± 16% of control). To determine whether the contractile response to CDS is mediated by Ca²⁺ channels, fundic strips were superfused with the Ca²⁺ channel blocker verapamil (1.0 µM). This treatment abolished the effect of CDS. The rat fundic strip is responsive to a variety of endogenous compounds. The contractile activity of partially-purified CDS might therefore be due to contamination by known bioactive substances. To determine whether the biological action of CDS is unique, one of several agonists, or CDS, was applied as a bolus to fundic strips. Appropriate antagonists were infused at increasing concentrations until complete blockade of the response to an agonist was achieved. Tested singly, none of the antagonists blocked the contractile effect of CDS. The combined effect of blocking agents was tested using a cocktail containing: atropine, des-Arg¹-Leu⁴-bradykinin, naloxone, phenoxbenzamine, propranolol, and theophylline (all at 1.0 µM), methysergide, piperidilicacci⁴-Va¹-Arg⁸-vasopressin (0.5 µM), TTX (0.1 µM), and saralasin (30 nM). This cocktail of antagonists had no effect on the contractile response to 1 Unit of CDS (85 ± 23% of control), indicating that the action of CDS is not due to biogenic amines, adenosine, angiotensin, opiates, or vasopressin. Specific antagonists are not available for neuropeptides and Substance P. However, cross-desensitization experiments showed that a constant infusion of agonist eliminated the effect of bolus doses of the same peptide but had no effect on the contract of CDS. In control experiments, the presence of methysergide to block serotonin receptors, yohimbine (5 µM) inhibited the effect of CDS (73 ± 4% of control), but not PGE2 (109 ± 4% of control). In conclusion, partially-purified CDS induces a rapid, dose-dependent contraction of the rat gastric fundus by a direct action on smooth muscle that is dependent upon Ca²⁺ channels. The contractile action of CDS appears distinct from known brain-gut transmitters, suggesting that CDS may be a unique substance.


Melatonin (MEL), the main hormone of the pineal gland, induces important effects, both in animals and humans. Such effects are modifications on sleep patterns, on thermoregulation, on locomotor activity and affective behavior between others. These effects suggest actions on central nervous system structures, mainly in limbic system. So far, we do not know if such actions are produced by the interaction between MEL and specific binding sites, or are mediated by other substances activated by MEL, because it has been shown that MEL is able to modify, both the production and the release of a great amount of hormones and neurotransmitters in several CNS sites. Obviously, to determine a possible direct action of MEL on brain cells, could be to analyze the changes in the electrical activity of these cells, after MEL administration. According to this idea, we decided to study the effects induced by MEL systemically administered on the multiunitary activity of several brain nuclei in unanesthetized freely-moving animals. Forty two Wistar male rats (180-230 g) were implanted with semi-purified CDS from bovine brain (Meeley et al., op cit). Smooth muscle preparations were suspended on force transducers with silk suture and superfused with oxygenated Krebs' buffer at 10 mL/min. Bolus applications of CDS had no effect on arterial (rat and rabbit aorta) or venous (dog and rabbit saphenous vein) vascular smooth muscle. The effects of CDS on rabbit jejunum and ileum were biphasic (contraction/relaxation) and variable. In contrast, CDS induced rapid, consistent, and dose-dependent contractions of the rat gastric fundus. In order to characterize the response to CDS, contractions were standardized to the peak tension elicited by prostaglandin E2 (PGE2, 200 pmoles). One Unit of CDS (equivalent to the amount of brain extract required to inhibit 7H-p-aminoclonidine binding by 50% under standard conditions) induced a contraction which was 82 ± 2% of that elicited by PGE2. To determine whether CDS acted directly on fundic smooth muscle or on intrinsic neurons, fundic strips were continuously superfused with tetrodotoxin (TTX, 0.1 µM). The response to CDS was not blocked, but slightly facilitated (peak tension = 113 ± 16% of control). To determine whether the contractile response to CDS is mediated by Ca²⁺ channels, fundic strips were superfused with the Ca²⁺ channel blocker verapamil (1.0 µM). This treatment abolished the effect of CDS. The rat fundic strip is responsive to a variety of endogenous compounds. The contractile activity of partially-purified CDS might therefore be due to contamination by known bioactive substances. To determine whether the biological action of CDS is unique, one of several agonists, or CDS, was applied as a bolus to fundic strips. Appropriate antagonists were infused at increasing concentrations until complete blockade of the response to an agonist was achieved. Tested singly, none of the antagonists blocked the contractile effect of CDS. The combined effect of blocking agents was tested using a cocktail containing: atropine, des-Arg¹-Leu⁴-bradykinin, naloxone, phenoxbenzamine, propranolol, and theophylline (all at 1.0 µM), methysergide, piperidilicacci⁴-Va¹-Arg⁸-vasopressin (0.5 µM), TTX (0.1 µM), and saralasin (30 nM). This cocktail of antagonists had no effect on the contractile response to 1 Unit of CDS (85 ± 23% of control), indicating that the action of CDS is not due to biogenic amines, adenosine, angiotensin, opiates, or vasopressin. Specific antagonists are not available for neuropeptides and Substance P. However, cross-desensitization experiments showed that a constant infusion of agonist eliminated the effect of bolus doses of the same peptide but had no effect on the contract of CDS. In control experiments, the presence of methysergide to block serotonin receptors, yohimbine (5 µM) inhibited the effect of CDS (73 ± 4% of control), but not PGE2 (109 ± 4% of control). In conclusion, partially-purified CDS induces a rapid, dose-dependent contraction of the rat gastric fundus by a direct action on smooth muscle that is dependent upon Ca²⁺ channels. The contractile action of CDS appears distinct from known brain-gut transmitters, suggesting that CDS may be a unique substance.
EVIDENCE THAT AT LEAST TWO GENES ENCODE MONOAMINE OXIDASE

464.1

WITH ALTERNATE SPLICING OF A COMMON PRECURSOR RNA. Northern

Northern blot hybridization with one of the cDNA clones which lines at the 5' end of the mRNA reveals a 3.7 kb poly A+ mRNA. The amount of this mRNA species in various tissues corresponds to levels of MAO-A enzyme activity. Human cDNA clones of MAO have also been isolated from placenta and liver libraries using the bovine cDNAs as probes. Preliminary DNA sequencing data suggest that these clones are at least 90% homology with scattered differences. These differences do not appear to arise from alternate splicing of a common precursor RNA. Northern blot hybridization with one of the cDNA clones which lines at the 5' end of the mRNA reveals a 3.7 kb poly A+ mRNA. The amount of this mRNA species in various tissues corresponds to levels of MAO-A enzyme activity. Human cDNA clones of MAO have also been isolated from placenta and liver libraries using the bovine cDNAs as probes. Preliminary DNA sequencing data suggest that these clones are at least 90% homology with scattered differences. These differences do not appear to arise from alternate splicing of a common precursor RNA.

MAO-B has been used to screen bovine adrenal medullary cDNA libraries. Two overlapping cDNA clones which span a 3.1 kb region have been isolated. Northern blot hybridization with one of the cDNA clones which lines at the 5' end of the mRNA reveals a 3.7 kb poly A+ mRNA. The amount of this mRNA species in various tissues corresponds to levels of MAO-A enzyme activity. Human cDNA clones of MAO have also been isolated from placenta and liver libraries using the bovine cDNAs as probes. Preliminary DNA sequencing data suggest that these clones are at least 90% homology with scattered differences. These differences do not appear to arise from alternate splicing of a common precursor RNA.

MAO-A and MAO-B have been described. Both forms are non-specific, insensitive to specific inhibitors (clorgyline, 0.01-10.0 µM for MAO-A and deprenyl, 0.1-10.0 µM for MAO-B). In order to visualize putative transmitters and modulators capable of stimulating either of the two forms of MAO, if any, in enteric serotoninergic neurons is not clear because little MAO-B is found in the cell bodies of these neurons. MAO-B is found in many enteric neurons that are not monoaminergic, but peptidergic. The role of MAO in these neurons remains to be determined.

Supported by NIH grant NS20530.

LOCATION OF MONOAMINE OXIDASE (MAO) TYPES A AND B IN THE ENTERIC NERVOUS SYSTEM OF THE GUINEA PIG SMALL INTESTINE. J. Sherman*, J. Pintar, and MD. Oershon (Spon. D. Coulter). Department of Anatomy and

464.3

Department of Pharmacology and Therapeutics, State University of New York at Buffalo, School of Medicine, Buffalo, NY 14214.

Phenal sulphotransferase (PST) catalyzes the transfer of a sulphonate moiety from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to a variety of catechol and phenolic acceptor molecules. Human placental cDNA coding for MAO-B containing cells, in cat central nervous system. J. Bak, F. Denaro, J.S. Schneider, R.B. McCauley, and F.W. Marquardt. University of California, Los Angeles, CA 90024, Dept. Ped., and Infectious Dis., UCSF Med. Cent., San Diego, CA 92103 and Dept. Pharmacol., Wayne State Univ. School of Medicine, Detroit, MI 48201. Little is known concerning the cellular and regional ultrastructural localization of MAO-B in the intact cat brain. Cats were anesthetized and perfused transcardially with either 4% paraformaldehyde (PF) in phosphate (pH 6.0) or borate (pH 11.0) buffers (for light microscopy) or with 4% PF in phosphate buffer (pH 7.4) plus 0.1% to 0.2% glutaraldehyde for electron microscopy. All tissue was vibratome cut (30 to 50 µm) and processed for EM. MAO-B was localized to neuronal somata and glial elements in cat brain. While MAO-B positive neurons were found in the hypothalamus, raphe system, locus coeruleus and dorsal pontine tegmentum, no labeled neurons were found in ventral mesencephalic dopamine regions. Labeled glial cells were found in almost all areas of brain. There was also a significantly greater density of MAO-B (glial) cells in the substantia nigra (SN) than in other nearby catecholamine cell groups. At the ultrastructural level, MAO-B was found in astrocytic footplates adjacent to terminals containing synaptic vesicles in the substantia nigra. MAO-B was also found in astrocytic footplates adjacent to dense-core vesicles. The localization of MAO-B in certain neural elements (e.g., neuronal somata, dendrites, boutons and glial cells) in various brain regions should help to clarify the role of this enzyme in influencing neurotransmitter function. Supported in part by grants USHS MH41645 and DE7282.
COCaine AND REGIONAL Monoamine UTILIZATION IN MICE Brain. M.G. BadfieId and C. HeU, Neurochemistry Research Laboratory, Section on Neuropathology, Department of Pathology, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA 23298.

Cocaine inhibits the uptake and enhances the release of central monoamines. This prolongs the activity of neurotransmitters at the synapse and may account for much of cocaine's action on the central nervous system. The present study was undertaken to see if cocaine produces selective changes in utilization of serotonin, dopamine, and/or acetylcholine, depending on the brain region and neurotransmitter system examined.

In the present study adult male ICR mice were given saline or 10 mg/kg cocaine i.p. 1/2 hr before sacrifice. The mice were sacrificed on the basis of evidence for specific changes in the utilization of neurotransmitters in each brain region. Controls and data from previous studies were used to determine conditions that produced changes greater than or equal to 2 SD above or below the control.

The drugs used were cocaine hydrochloride and saline. The control brain regions were: the rostral and caudal ventral tegmental area, ventral pallidum, hypothalamus, cerebellum, locus ceruleus, and the forebrain (prefrontal cortex, striatum, and median eminence).

The results indicate that cocaine, in doses of 10 mg/kg, produced increased utilization of serotonin in the rat hypothalamus and increased uptake of dopamine in the rat caudate. These alterations were revealed by changes in the tissue levels of the endogenous monoamines. The increased uptake of dopamine, but not serotonin, was accompanied by an increase in the tissue concentration of dopamine metabolites. These results are consistent with the hypothesis that cocaine produces a sympathomimetic effect on the central nervous system.

In another experiment, the effects of cocaine on the utilization of serotonin and dopamine in the rat hypothalamus were examined. The results indicated that cocaine produced a significant increase in the utilization of serotonin and dopamine in the hypothalamus. The increases in utilization were greater than those observed in the forebrain and were of the same magnitude as those previously reported.

The results of these experiments support the hypothesis that cocaine produces selective changes in the utilization of neurotransmitters in the brain. These changes may be responsible for the behavioral effects of cocaine, such as increased alertness and increased motor activity.

Further studies are needed to determine the mechanisms by which cocaine produces these changes in the utilization of neurotransmitters. It is possible that cocaine acts by altering the activity of neurotransmitter systems or by altering the activity of enzymes involved in the metabolism of neurotransmitters.

The findings of these studies suggest that cocaine produces selective changes in the utilization of neurotransmitters in the brain. These changes may be responsible for the behavioral effects of cocaine and may be involved in the development of tolerance and dependence.

These results also suggest that cocaine may be useful as a research tool for studying the effects of neurotransmitter systems on behavior.

References


464.9 THE DETERMINATION OF FREE HOMOVANILIC ACID (HVA), (3-METHOXY-4-
HYDROXYPHENYL) ETHYLENE GLYCOL (MHPG), AND VANILLANILIC ACID

Many studies to date have demonstrated the usefulness of measuring catecholamine metabolites found in biological fluids in studies of psychiatric disorders such as mania and schizophrenia. Several of these studies have indicated that differences in levels of these compounds are due to factors such as diet, exercise and diurnal variation. Our laboratory has been interested in levels of the dopamine metabolite HVA, as well as the noradrenergic metabolites MHPG and VMA, and whether or not these compounds could be measured in saliva. In addition, we wanted to know if changes in saliva levels of the catecholamine metabolites would directly correlate with levels seen in plasma. Saliva is a bodily fluid that can be obtained in an easy and convenient manner, providing repeated measures of catecholamine metabolite levels that would be especially useful in clinical studies involving children.

The determination of the methoxylated catecholamine metabolites entails extraction of the compounds from saliva with ethyl acetate, following acidification and addition of acetic chloride. The ethyl acetate fractions are combined and evaporated to dryness. The resulting residues are resuspended in pH 3.0 buffer and injected into a high performance liquid chromatography system coupled with coulometric electrochemical detection. The methoxylated compounds are separated and quantitated using a gradient elution procedure similar to the method that we have recently reported (Gerhardt, G.A., Drebign, C.J. and Freedman, R., Anal. Chem., 58: 2879, 1986).

Initial results support that levels of salivary catecholamine metabolites correlate with plasma levels. Correlations range from 0.95 for HVA to 0.65 and 0.57 for MHPG and VMA respectively. (Supported by USPHS grants MH-38321 and DA-02429, and VA Medical Research Service.)

Our data suggest that under the present experimental conditions, approximately 30% of the extracellular DA pool was stable over a 2 hour period (fmo/mg 10 min collection: DA = 71 ± 14; 3-MT = 23 ± 1.7). DOPAC = 17,700 ± 400; HVA = 10,200 ± 200). Amphetamine (5 mg/kg ip) caused a rapid increase (1500%) in DA levels within 20 min after administration and this effect was followed by increases in DA levels within 20 min after administration. Under both basal and d-amphetamine-stimulated conditions, the 3-MT pool was maintained at approximately 30% of the extracellular DA pool. Dopamine levels showed no significant change over time and were not significantly altered by amphetamine.

These findings suggest that the 3-MT is an accurate index of synaptic DA release.

Supported by NIBRS-7520622.

464.10 DETERMINATION OF FREE AND BOUND METABOLITES OF NOREPINEPHRINE IN RAT BRAIN BY HPLC WITH SERIAL OXIDATIVE-REDUCTIVE ELECTROCHEMICAL DETECTION. D.K. Dean, G.H. Gerhardt, H. Wochneroff. Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309 and Dept. of Psychiatry, Univ. of Colorado Health Sci. Ctr., Denver, CO 80262

High performance liquid chromatography coupled with electrochemical detection is an efficient means for the concurrent quantification of monoamines and their metabolites in brain. Unfortunately, measurement of the principal metabolites of norepinephrine, dihydroxyphenylglycol (DHPG) and 3-methoxy-4-
hydroyphenyl glycol (MHPG), is difficult because they are extensively sulfated in most rat brain regions. In addition, other electroactive species coelute with these compounds, and interfere with the measurement of even their free quantities. We describe here methods for the measurement of free and total DHPG and MHPG in rat brain using serial coulometric detection. Frozen samples of anterior cortex, hippocampus and cerebellum were sonicated in 0.3M acetate pH 5 buffer and centrifuged at 17000g for 20'. The supernatants were injected directly on a Beckman 1C8 reverse phase column (15cm x 4.6mm, 5m packings). A pH 4.00 citrate-acetate sodium nitric acid/HzO mobile phase was used at a flow rate of 1.6 mI/min. Column output was passed serially through three coulometric detectors (5020 Guard Cell and 5011 Dual Cell, with Model 5100A Coulachem Controller, ESA, Inc.): the first detector serves to oxidize compounds of interest at +3.5 +5.0V the second to screen out easily reduced interferences at 0 -10V, and the third for peak quantification in the reducing mode at -5 -40V. With careful adjustment of cell potentials, DHPG and MHPG can be accurately measured at the third detector. This detector configuration permits the simultaneous determination of norepinephrine, dopamine and its metabolites DOPAC and HVA, serotonin and its metabolites 5HIAA and 5-hydroxytryptophan and uric acid. Finally, following treatment with sulfatase (S3009 Sigma, 4mg/ml sample volume) for 12 hours at 35°C a sample aliquot can be injected directly for determination of free plus sulfated DHPG and MHPG.

Thus, the monoamine neurotransmitters, their precursor amino acids and their metabolites, including the free and bound metabolites of norepinephrine, can be determined in a single brain sample.

Supported by NSF-NSB 8520622.

464.11 REGIONAL ANALYSIS OF RAT BRAIN DOPAMINE AND SEROTONIN METABOLISM AFTER AN ACUTE LOW DOSE OF ETHANOL. Steven A. Siga*, Bryan R. Yamamoto* and Martin D. Schechter* (SPON: M. Naglejane). Dep. of Pharmacology, Grinnell College, Grinnell, Rochester, NY 14618 and 2 Dept. of Pharmacology, Nova College of Medicine, Rootstown, OH 44272.

Research on the effect of high doses of ethanol (greater than 2 g/kg) on rat brain dopamine and serotonin metabolism has resulted in variable results ranging from increases to decreases in metabolism, to no discernable change. The dose of ethanol in these studies (0.2 g/kg) elicits a sedative-hypnotic type reaction in laboratory animals which may not truly represent the reinforcing component thought to be responsible for ethanol abuse. Therefore we have chosen a low dose of ethanol (0.6 g/kg IP), shown in our laboratory animals which may not truly represent the reinforcing activity in the negative chemical ionization mode.

The baseline levels of DA and its metabolites were stable over a 2 hour period (fmo/mg 10 min collection; DA = 71 ± 14; 3-MT = 23 ± 1.71 DOPAC = 17,700 ± 400; HVA = 10,200 ± 200). Amphetamine (5 mg/kg ip) caused a rapid increase (1500%) in DA levels within 20 min after administration and this effect was followed by increases in DA levels within 20 min after administration. Under both basal and d-amphetamine-stimulated conditions, the 3-MT pool was maintained at approximately 30% of the extracellular DA pool. Dopamine levels showed no significant change over time and were not significantly altered by amphetamine.

Our data suggest that the 3-MT is an accurate index of synaptic DA release.
464.13 THE EFFECT OF BUSPIRONE AND ITS METABOLITE ON SEROTONIN, DOPAMINE AND SEROTONIN turnov IN RAP BRAIN. Kenneth W. Ferry and Ray W. Fuller. Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46225.

Buspirone is an anxiolytic drug which is rapidly metabolized to 1-2-pyridylmethylpipеразине (1-PP) [Caccia et al., Pharmacology 33:46-51 (1986)]. Since the metabolite was present in brain at a concentration higher than that of buspirone, the possibility of a role in therapeutic or other actions of buspirone has been considered. Our purpose in the studies reported here was to compare the biochemical changes produced by buspirone and by its metabolite, 1-PP.

Buspirone caused a dose-related decrease in hypothalamic 5-hydroxytryptamine (5-HT) concentration at doses of 1-10 mg/kg s.c. in rats. At a dose of 3 mg/kg the decrease was greatest at 1 hour (-35%), with a rapid return to near control levels by 6 hours. The decrease in 5-HT was apparent to a reduced turnover of serotonin since the accumulation of 5-hydroxytryptophan after decarboxylase inhibition by MHPG was suppressed at doses of buspirone from 0.3 to 3 mg/kg s.c. Buspirone also increased the concentrations of the dopamine metabolites homovanillate and 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillate and measured in whole brain and in striatum, and increased the accumulation of DOPA in striatum after decarboxylase inhibition. The dose response and time course were similar to those described for both cases MHPG-sulfate levels had returned to normal by 6 hours. At a 3 mg/kg dose the increase reached 5-hydroxytryptophan after decarboxylase inhibition by NSD 1015 was reduced turnover of serotonin since the accumulation of 5-HT was transiently decreased by 42% (10 mg/kg MDMA, 1-2 hrs). MDMA caused a dose-related decrease in hypothalamic 5-HT, which began to recover at 3 mg/kg s.c. in rats. At a dose of 3 mg/kg the decrease was 8 mg/kg MDMA, 1-2 hrs) slightly but non-significantly increased

464.14 EFFECTS OF HYPOXIA ON CATECHOLAMINE METABOLISM IN BRAIN SUPERIOR CEREBRAL GANGLION. B. Dinger, G.-W. Cheng and R.J. Pidocke. Dept. Physiol., School of Medicine, Univ. Utah, Salt Lake City, Utah 84118.

In previous studies we demonstrated that hypoxia reduces the content of substance P (SP) and increases glucose utilization in superior cerebral ganglia (SCG) which were either chronically decentralized or exposed to reduced O2 in vitro (Dinger et al., Neurochem. Abstr, 22:1345 (1986)). Parallel studies of the neural chemonomy of the carotid body revealed similar changes in SF content and glucose consumption, while experiments with a nodose sensory plexus isolated showed no effect on SP levels or intermediary metabolism. These findings suggest that the SCG contains a relatively high concentration of cells sensitive to those of the carotid body. In the present study, we have evaluated changes in catecholamine (CA) metabolism induced in the SCG by hypoxia.

Deaerated SCG were preincubated for 40 min in Tyrode's solution equilibrated with 100% O2 (37°C). The ganglia were transferred to vials containing media equilibrated with either 100% O2, 4O2:2% O2 or 20% O2, where superfusion continued for 10 min (37°C). Incorporated was determined in 0.2% perchloric acid (PCA). The effects of hypoxia on CA metabolism were also studied in slices of rabbit carotid cortical. Slices of rabbit cortical was quantified using reverse phase HPLC with electrochemical detection.

The level of dihydroxyphenylalanine (DOPA) was low in ganglia incubated in 100% O2 media (0.09 mg/kg of tissue). DOPA content increased 7.4% and 1.4% following exposure to 4O2:2% O2 and 20% O2 media, respectively. In contrast, the level of DOPA in 5HT nerve terminal areas (caudal brainstem) and was dose-dependent (3-30 mg/kg). In chronic treatment are not caused by reduced availability of neurotransmitter bioavailability and potency in vivo. Supported by USPHS grants NS12636, NS07938.
464.17 SODIUM-DEPENDENT UPTAKE OF L-KYNURENINE INTO SLICES FROM RAT BRAIN. C. Speciale1, W. A. Turski1, N. Brookes2 and R. Schwartz2.1, 2Maryland Psychiatric Research Center, Baltimore, MD 21228 and 2Dept. Pharmacology, Univ. of Maryland School of Medicine, Baltimore, MD 21201.

L-Kynurenine (KYN) is the bioprecursor of both the neuroinhibitory compound kynurenic acid and the neuroexcitatory and excitotoxic brain metabolite quinolinic acid. KYN is known to be able to cross the blood-brain barrier (Neurochem. Rev. 5, 223, 1980) but the process responsible for its subsequent transport into brain cells has as yet not been directly examined. We report here the presence, in rat brain tissue, of specific uptake mechanisms for KYN.

In routine experiments, two 500 μm thick slices from adult rat striatum were incubated for one hour at 37°C in an oxygenated culture well containing 1 ml Krebs-Ringer buffer (pH 7.4) and 800 μM (0.2 Ci/ml) [3H]-KYN (specific activity 8.9 Ci/mmol). Following incubation, slices were carefully rinsed, dissolved and their radioactivity measured. The assay conditions, established in pilot experiments, assured the conduct of the study in the linear range for both tissue amount and incubation time. 3H-KYN was taken up into slices at a ratio of approximately 5:1 (tissue:medium). Incubation in no Na+ buffer yielded 32% of the uptake found in slices incubated with Na+.

Specificity of the uptake process was demonstrated by the inability of glutamate, aspartate and several metabolites of the kynurenine pathway (at 1 mM) to inhibit the Na+-dependent uptake of 3H-KYN. By contrast, putrescine, melatonin and vitamin C (1 mM) failed to affect the uptake process. Following incubation, slices were carefully rinsed, dissolved and their radioactivity measured. The assay conditions, established in pilot experiments, assured the conduct of the study in the linear range for both tissue amount and incubation time. 3H-KYN was taken up into slices at a ratio of approximately 5:1 (tissue:medium). Incubation in no Na+-buffer yielded 32% of the uptake found in slices incubated with Na+.

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464.2 NEUROCHEMICAL CORRELATES OF TRIGEMINAL, POST-TRAUMATIC, AND
neurochemistr[y of trigeminal pain. The release of
neuropeptides into the cerebrospinal fluid (CSF) can be quantified
by measuring their concentration in samples of CSF obtained
from patients with trigeminal neuralgia (TG). The specific
neuropeptides measured include somatostatin (SRIF), substance P,
and calcitonin gene-related peptide (CGRP). The concentrations of
these neuropeptides in the CSF were found to be significantly
different in patients with TG compared to healthy controls.

Methods: CSF samples were obtained from patients with TG
and healthy controls. The concentrations of SRIF, substance P,
and CGRP were measured using ELISA technology.

Results: The concentrations of SRIF, substance P,
and CGRP were found to be significantly higher in
the CSF of patients with TG compared to healthy controls.

Conclusion: The findings suggest that the release of
specific neuropeptides into the CSF may be a biomarker
for trigeminal pain. Further studies are needed to
elucidate the mechanisms underlying this release.

464.22 SEQUENTIAL METABOLISM OF ANGIOTENSIN II AND III BY MEMBRANE
PEPTIDASES FROM RAT BRAIN. J.M. Hansen**1, J.W. Harding, and R.H. Abdolv.
This study examined the metabolism of [125I]angiotensin II
conversion of [125I]AliII to [125I]AliI was markedly inhibited by All, AliI, and (3-8)AliI. In contrast,
[125I]AliII was preferentially metabolized by one or more carboxypeptidases whereas the metabolism of [125I]AliII was clearly different than that of applied
[125I]AliII. It is likely that the two pathways reflect differences in substrate concentration and enzyme affinity. The specific angiotensinase activity may modulate the physiological effects of angiotensin peptides.

Increases in brain prostaglandin (PG) levels may contribute to some of the functional and morphological abnormalities that are observed to be caused by exposure to cold injury in cats (Billa, et al., J Neurochem. 37:892-896, 1981). In order to determine whether similar increases in prostaglandin levels occur in a rodent model of cold injury, rats were anesthetized with pentobarbital and prepared for injury (Dixon, et al., J Neurosurg. 46:654-662, 1977). Post-traumatic hind limb convulsions were observed in 4 of the 7 rats during the 15 minutes before sacrifice. These animals were analyzed separately.

Figure. Effects of fluid percussion brain injury on hemispheric and thalamic PG levels (s). Data are expressed as mean ± SE.

These transient increases in PG levels are consistent with a recent report of elevated PG synthesis in a different rodent model of traumatic brain injury (Hoshii, et al., J.Cerebral Blood Flow & Metab. 7:58-63, 1987). Supported by NIH grant NS19355.

465.2 PROXIMAL NEUROPLASTIC SWELLINGS AND MITOCRIONAL ALTERATIONS, FOLLOWING HIGH Dose CARBON DISULFIDE ADMINISTRATION. B.B. Gold*, I.K. Griffin*, S.O. Andrews and L. Zafi*, Rutgers University College of Pharmacy, Piscataway, NJ 08854; The Johns Hopkins University School of Medicine, Baltimore, MD 21239 and NIMHD, Bethesda, MD 20892.

Distal neuroplasmic swellings are observed in a variety of experimental conditions. The precise effects of exposure to cold Injury in rats (Billa, et al., J Neurochem. 37:892-896, 1981), a rodent model of cold injury, was studied. Post-trauma hind limb convulsions were observed in 4 of the 7 rats before sacrifice. These animals were analyzed separately.

Figure. Effects of fluid percussion brain injury on hemispheric and thalamic PG levels (s). Data are expressed as mean ± SE.

465.3 IMMUNOELECTRON MICROSCOPIC INVESTIGATION OF COLD INJURY IN THE GERbil BRAIN. M. Maeda*, F. Akai*, S. Nishida* and T. Yanaqihara. Dept. of Neurology, Mayo Clinic, Rochester, MN 55905 and Dept. of Pathology, Kyoto University, School of Medicine, Osaka, Japan.

In cold injury, an extensive vasogenic cerebral edema with extravasation of albumin occurs. In the present investigation, we studied the distribution, particularly intracellular localization, of albumin by electron microscopical immunocytochemistry and correlated the finding with morphological alterations. A cold injury was produced in the parietal cortex of the gerbil. Immunocytochemical procedure was carried out with a goat anti-rabbit antibody, which was produced by using an antiserum for gerbil albumin and the peroxidase-antiperoxidase complex. Ultrastructural investigation was focused on the abnormal neurons.

Thirty minutes after cold injury, extravasated albumin was already observed in the extracellular space and within a few neuronal cell bodies and dendrites. In some dendrites, the distribution of microtubules was irregular and the number was reduced. The positive reaction for albumin corresponded most closely to the distribution of polyribosomes. The proportion of responding phenotypes and 3) whether the PR is limited to the CNS but are of hematopoietic origin; and 3) in contrast, the PR in distant but related regions remains unclear. However, it is interesting to note that their location in mitochondria coincides with the copper/zinc isozyme of superoxide dismutase and that inhibition of copper/zinc superoxide dismutase has been implicated in the genesis of CD neurotoxicity. Supported by NIH grant ES 04708.


Cellular proliferation has long been recognized as a consequence of brain injury. However, due to the limitations of in vivo methods, the course of events and identification of responsible cells have been largely uncertain. In this study, we used two approaches to study the kinetics and the extent of the proliferative response (PR) in vitro by using DNA incorporation 2) the proportion of responding phenotypes and 3) whether the PR is limited to the CNS but are of hematopoietic origin; and 3) in contrast, the PR in distant but related regions remains unclear. However, it is interesting to note that their location in mitochondria coincides with the copper/zinc isozyme of superoxide dismutase and that inhibition of copper/zinc superoxide dismutase has been implicated in the genesis of CD neurotoxicity. Supported by NIH grant ES 04708.

The significance of the metal deposits remains unclear. However, it is interesting to note that their location in mitochondria coincides with the copper/zinc isozyme of superoxide dismutase and that inhibition of copper/zinc superoxide dismutase has been implicated in the genesis of CD neurotoxicity. Supported by NIH grant ES 04708.
465.5 CONSTRUCTION OF THE SQUID GIANT AXON AFTER TRANSSECTION. T. E. Gellman. Laboratory of Neurobiology, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health at the Marine Biological Laboratory, Woods Hole, MA 02543.

After transection both ends of a cut axon reform a high resistance seal (Heirst et al, Brain Res. 19:183-198, 1983). The function of this constriction is to maintain a low extracellular ionic calcium and the activation of a calcium dependent phospholipase (Tawo H. and Kono M., J. Neurosciences 5:1626-1632 1985). In our recent study (in prep) we showed that at the cut end, exposure of the cut end to the extracellular ionic concentrations may also induce changes in the cytoskeletal proteins and the size and shape of the axon which are in part maintained by the cytoskeletal proteins. In the squid giant axon transections led to a softening of the exposed axoplasm and a pinching off (constriction) of the cut end. Constriction occurred when the axon was cut in the presence of external medium (an artificial squid blood), but failed to occur when the axon was cut in the presence of internal medium (composed of the ions normally present inside the axon). This difference in the amounts of constriction was largely due to the different cation composition of the two media. Specifically constriction required the Na concentrations of calcium ions, and the relatively low potassium to sodium ratio present in the external medium. Softening of axoplasm appeared to be necessary for constriction since constriction only occurred under conditions which induced axoplasmic softening. Axoplasmic softening was not sufficient for constriction, however, since constriction could be inhibited (for example by reducing ATP levels with dinitrophenol) without inducing axoplasmic softening. These results suggest that exposure of the cytoskeletal proteins to the extracellular ionic concentrations induces cytoskeletal changes (like axoplasmic softening and perhaps a contraction of some of the cytoskeletal proteins) that contribute to the response and perhaps repair of a damaged axon.

465.6 ENDOTHELIAL CELL CHANGES IN RESPONSE TO EXPERIMENTAL HYPERTENSION OR DIABETES SINGLY OR IN COMBINATION. P.A. Grady. Dept. Neurology, University of Maryland Sch. of Med., Baltimore, Maryland.

This study tests the effects of hypertension and diabetes singly and in combination on the cerebral blood vessels. Adult male spontaneously hypertensive rats (SHR) were made diabetic by injection of streptozotocin (45 mg/kg). Adult male Wistar-Kyoto rats were used as controls and also served as control groups. Animals were maintained for up to three months with either or both of these factors. Blood pressures were monitored weekly on the caudal artery using the indirect tail-cuff method. Blood glucose was monitored weekly using the o-toluidine method.

After three months in experimental groups, animals were anesthetized and prepared for recordings using the left ventricle of the heart with fixative. Major cerebral arteries were removed and prepared for scanning electron microscopy (SEM). Comparison of vessel changes in response to these experimental risk factors was made among the hypertensive diabetic rats, hypertensive nondiabetic rats and normotensive diabetic rats at the same duration of risk exposure.

After three months of exposure to both risk factors in combination, the major cerebral arteries showed distinct signs of alteration of luminal surfaces. Endothelial surfaces were swollen or frothy in appearance with areas of frank endothelial injury superimposed on the irregular surfaces. Systemic vessels were affected more severely, but the pattern of abnormalities was essentially the same. Examination of age-matched animals with the simple risk factor of hypertension for the same duration revealed frank endothelial injury and subtle restructuring of endothelial surfaces, but luminal surfaces did not appear swollen as in the diabetic hypertensives. The vessels of age-matched animals with diabetes alone for the same period of time showed a distinctely cobbled or swollen appearance, but the absence of frank endothelial injury. These changes were consistent.

In conclusion, these findings provide evidence which strongly supports the idea that each of these important risk factors for the development of cerebral vascular disease and stroke results in a different type of injury or structural change in the early periods which we have studied, and that the combination of these two risk factors, hypertension and diabetes, results in lesions that are different in nature from those seen in vessels exposed to each risk factor singly. These structural changes provide a basis for altered hemodynamic patterns and increased thrombogenic potential. These findings are important in our further understanding of the mechanisms of early vascular changes resulting from these two major risk factors for developing a stroke. Supported in part by NIH grant NS 16332.


We have used in vivo subcutaneously implanted meningiomas in nude mice to evaluate growth of these tumors during hormonal manipulation. Human meningioma tissue was obtained from two patients. Portions of the tumor were used for estrogen and progesterone binding protein determinations by Scatchard analysis. The remaining portions of each tumor were divided into equal fragments of 3x3x3 mm. These were implanted over the left scapula in a subcutaneous pocket using a minute weight of 35-45 mg, allowing intraperitoneal choral hydrate anesthesia. The 20 mice used for each experiment were divided into 4 groups of 5 mice. These groups received oral estrogen or progesterone in doses of 7.5 mg/kgm/day or the antiprogestational compound RU38468 at a dose of 10 mg/kgm per day. Five mice served as controls. The meningioma volumes were determined by a formula V=W^2L/2 where V represented the total volume, W the width and L the length by Anthropic measurements. These tumors were measured 2 weeks following implantation, then on a monthly basis for 3 months. Tumor volumes were compared at the beginning and end of the experiment and a growth index (GI) was calculated as GI=Vf/Vi with Vf representing the volume determination at the end of the experiment and Vi representing the volume of the tumor 1 season. Results were compared by student "t" tests for unpaired data.

The initial tumor tested was found to have progesterone binding proteins in concentrations of 30.6 nM/mg cytosol protein and no estrogen binding proteins. Growth indices of 1.25 in control animals and 1.49 in progesterone treated animals were significantly greater than the growth index of 0.89 in the RU38468 treated animals. Animals treated with beta estradiol showed no significant changes in growth. The second tumor was also found to have progesterone binding proteins at a concentration of 44.8 nM/mg protein. However, in this tumor growth of the meningiomas was not seen and growth indexes were less than 1 for all groups. There was no statistically significant difference in the growth indexes of all three groups compared to one another. These results suggest that in certain meningiomas, hormonal manipulation may be of value in controlling tumor growth. At this time there is no known method of determining which meningiomas will respond to hormonal manipulation. Further in vitro and in vivo trilal trials may lead to a better understanding of antiprogestational compounds in the treatment of inoperable or recurrent meningiomas.

465.8 IN VIVO EFFECTS OF ESTROGEN, PROGESTERONE AND THE ANTIPROGESTERONE RU38468 ON MENINGIOMAS IN NUDE MICE. R. M. Port*, J. R., D. K. Viett. Division of Neurosurgery, University of Iowa Hospitals, Iowa City, Iowa 52242.

Experimental diabetic neuropathy is characterized by reduced peripheral nerve conduction velocity which is manifested as early as two weeks after induction of diabetes, diminished Na+ K+-ATPase activity and structural abnormalities of the axo-glial junction (Sima et al (1986) J. Neurosurgery. 5:1626-1632). These changes are consistent with the different cation composition of the two media. Specific changes, we have used x-ray microprobe analysis to determine the concentrations of elemental Na,K,P,Cl and Ca in subcellular compartments of sciatic nerve from normal and diabetic rats. Axonal cytoplasm, mitochondria, myelin and extra-axonal space were examined following preparation of frozen, ultrathin, unfixed transverse and longitudinal sections. Upon analysis of transverse sections, normal axoplasm exhibited (in µmoles/g dry weight) low Na (96 ± 10) and high K (120 ± 65) levels. The contents of these elements in extra-axonal space were 1697 ± 179 and 375 ± 119. In axoplasm from nerves of rats that had been diabetic for 10 weeks, Na showed no significant change (117 ± 14) but was substantially elevated (202 ± 32) after 2 weeks. K levels were not significantly altered, but Ca tended to increase. Mitochondria from diabetic rat nerve displayed elevated Na and Ca and decreased K, whereas the elemental content of myelin was not significantly altered. The concentrations of Na levels in diabetic nerve were much less pronounced than those observed either in ischemic nerve or following axotomy. Limited examination of longitudinal sections suggested that Na levels were concentrated at the nodes of Ranvier and decrease progressively with increasing distance from the node. These data support the conclusion that intra-axonal Na accumulation occurs in chronic experimental diabetes at a time well after the reported onset of decreased conduction velocity. The possibility exists that the increased Na levels are associated with the disappearance of normal axo-glial junctions. (Supported by NIH grants DK30577 and ES03830).
465.10 AN in vivo 31P NMR STUDY OF THE ACCUMULATION OF PHOSPHOETHANOLAMINE BY NEUROBLASTOMA CELLS IN A/J MICE AS A FUNCTION OF THEIR LOCATION IN THE TUMOR: P. Ogawa, R. Chopp, E. Suwita, D. M. Lee (SPON: R. Ogawa). The organophosphorus compound OPIDN has not been established, Ca++-calmodulin dependent peripheral distal axonopathy. Although the pathogenesis of the neuropathy supported by in vivo 31P NMR spectra of the cortex of these tumors showed a large phosphononoester and Pi peaks, but essentially no phosphinate peak. The Pi peak indicated the cellular pH was 7.2 to 7.0. The major components of the phosphonoester peak was PEO as determined by the chloroform-methanol extract of the tumors. The spectra of a whole tumor varied in extent with the age of the tumor and with the metabolic conditions of the animal. Variation appeared in the amounts of phosphonoester, Pi and phosphate and also in the pH values. We have not yet been able to obtain the spectra of the slurry because of the spatial complexity and the small volume fraction of the neuroblastoma cells in the slurry relative to the cortex. We are interested in finding out which anatomic site of a tumor (the cortex of the slurry) contains the dividing cells for the growth of the tumor.

465.11 FUNCTIONAL SIGNIFICANCE FOR THE SEQUENCE HOMOLOGY BETWEEN HIV IMMUNODEFICIENCY VIRUS AND NEUROLEUKIN. M. R. Lee, P. D. Ho, and M. E. Pinckard (SPON: M. E. Pinckard). Neurotrophic factors are important for the development and maintenance of the nervous system. Nerve growth factor (NGF) remains the best conserved region of the external envelope protein of the human immunodeficiency syndrome (AIDS). Neurological dysfunction, termed AIDS dementia complex, is common in AIDS and is due to infection of the brain by HIV. We have tested the hypothesis that an HIV antigen suppresses neuronal neurotrophic activity of NGF; (2) the inhibition is due to the external envelope region of NLK sequence homology is not involved with binding of HIV to CD4, but has no effect on the neurotrophic activity of NGF; (2) The inhibition is due to the external envelope region of NLK sequence homology potently neutralizes HIV infectivity. The homology with NLK is conserved in all HIV for which sequence information is available, and undoubtedly denotes strong selective pressure on HIV phenotypic expression. We now have experimental evidence which demonstrates the importance of this region for HIV infectivity. We find that a rabbit antiseraum made against a synthetic peptide from the gp120 region of HIV sequence homology potently neutralizes HIV infectivity in vitro. In addition, evidence from the laboratory of D. M. Martin (NIH) shows that introduction of a single point mutation into a potential glycosylation site within the envelope glycoprotein of the retrovirus unrelated to HIV, SAIDS-D, does not inhibit either NLK or NGF. We hypothesize that gp120 inhibits NLK due to sequence homology with toe factor.

465.12 INCREASED AUTOPHOSPHORYLATION OF CALMODULIN KINASE II AFTER TREATMENT WITH AN ORGANOPHOSPHORUS COMPOUND. E. Suwita*, D. M. Lee, W. M. Lapaud*, and M. R. Lee (SPON: M. R. Lee). Calmodulin dependent protease phosphorylation by Calmodulin Kinase II has been found to play an important role in the development of the neuropathy. In this report, the activity of Calmodulin Kinase II was further investigated in chickens given a single oral dose of 0 or 750 mg/kg and killed after 1 day following treatment. Brains were homogenized in 10 mM PIPES, pH 6.9, 10 mM EDTA and 10 mM EDTA (1 mM/mg) and centrifuged at 100,000 x g for 1 hr. The cytosol from individual animal was passed through 200 mm filters following a single administration of the compound. The condition is characterized by an ataxia which progresses to paralysis concurrent with a central-peripheral distal axonopathy. Although the pathogenesis of the neuropathy has not been established, Ca++-calmodulin dependent peripheral distal axonopathy. Although the pathogenesis of the neuropathy supported by in vivo 31P NMR spectra of the cortex of these tumors showed a large phosphononoester and Pi peaks, but essentially no phosphinate peak. The Pi peak indicated the cellular pH was 7.2 to 7.0. The major components of the phosphonoester peak was PEO as determined by the chloroform-methanol extract of the tumors. The spectra of a whole tumor varied in extent with the age of the tumor and with the metabolic conditions of the animal. Variation appeared in the amounts of phosphonoester, Pi and phosphate and also in the pH values. We have not yet been able to obtain the spectra of the slurry because of the spatial complexity and the small volume fraction of the neuroblastoma cells in the slurry relative to the cortex. We are interested in finding out which anatomic site of a tumor (the cortex of the slurry) contains the dividing cells for the growth of the tumor.
DIRECT EVIDENCE OF MEMBRANE MEDIATED CELLULAR DEGENERATION BY CHANGES IN THE INTRACELLULAR FREE CALCIUM, POTASSIUM, AND SODIUM CONCENTRATIONS IN DYSTROPHIC HAMSTERS. S.K. Bhattacharya, J.R. Lopez*, V. Sanchez*, A.J. Crawford, J.L. Vergara*, and F. Sreter*. University of Tennessee, Memphis; Instituto Venezolano de Investigaciones Cientificas, Caracas; University of California, Los Angeles; and Ion Biomedical Research Institute, Boston.

We have shown that CHF-146 strain dystrophic hamsters (DH) represent an experimental model for the study of muscular dystrophy (MD) because of the striking pathogenetic similarities in the biochemical abnormalities, cellular necrosis, ECC changes, and muscle mineralization and histopathology (Bhattacharya et al. Muscle & Nerve, 11:637, 1988). Changes in the concentrations of intracellular ions follow approximately the same course, but proceed more rapidly. Furthermore, in the mouse, some of the fibers degenerate more slowly than the majority, and some normal-looking fibers persist even at late stages. By supported grants from the NIH (NS-04945) and MMS (NS-1579).

ABSENCE OF CALCIFIC DEGENERATION IN THE CARDIAC AND SKELETAL MUSCLE OF C57BL/6-J2 DYSTROPHIC MICE AND THEIR RELEVANCE AS A MODEL FOR MUSCULAR DYSTROPHY IN HUMANS. Debra J. Jackson*, Alice J. Crawford and Syamal K. Bhattacharya. Surgical Research Laboratories, University of Tennessee, Memphis, TN 38163.

Because of hind limb deformity and neurogenic changes, C57BL/6-J2 dystrophic mice (DM) have been studied for muscular dystrophy. A study of human familial and sporadic cases of muscular dystrophy (DMD, Becker, and LGMD) in comparison with the CHF-146 strain dystrophic hamsters (DH) in comparison. Our studies show that CHF-146 mice resemble DM in terms of dystrophic pathology and calcification in the heart, whereas DH reveal both the skeletal and cardiac muscle changes. By supported grants from Am. Heart Assoc, and Varian Instrument Group of America.
DEPRESSION OF THE MONOSYNAPTIC REFLEX IN THE NEONATAL RAT SPINAL CORD BY DIFLUOROPROPIONIC ACETIC ACID: MECHANISMS FOR NERVOUS SYSTEM DESTRUCTION AND NEUROTOXICITY III

466.1 INACTIVATION OF p-GLUTAMYLGLYCINE SYNTHETASE AND GLUTAMINE SYNTHETASE BY OXYGEN RADICALS: IMPLICATIONS FOR NERVOUS SYSTEM DISEASE. Hina F. Schor* (SPON: P. Wade) Division of Child Neurology, University of Pittsburgh, Pittsburgh, PA 15213

Oxygen free radicals have been implicated in the toxicity of oxygen for the central nervous system and in post-ischaemic reperfusion injury seen after cardiac arrest and stroke. One physiological mechanism for detoxification of oxygen radicals is their reduction by reduced glutathione. On the other hand, oxygen radical damage is exaggerated in the presence of the excitatory neurotransmitter, glutamate. We present herein evidence for the inactivation of enzymes responsible for the synthesis of glutathione and for the conversion of glutamate to its inactive metabolite, glutamine, by oxygen radicals.

It has been long been thought that oxygen radicals deplete cellular glutathione by oxidizing it to its disulfide form. However, we have found that total (reduced plus oxidized) glutathione levels decrease in A/J mice given 6-hydroxydopamine, an oxygen radical generator. This implies that oxidation of reduced glutathione is not the sole mechanism of its depletion. We have recently found that the rate limiting enzyme for glutathione synthesis, p-glutamylglutamate synthetase, is inactivated by 6-hydroxydopamine at concentrations of the drug which are theoretically obtainable in animals. Inactivation of the synthetase by 0.67 μg/ml 6-hydroxydopamine is complete by 2 hours at pH 7.0 and 37°C. The inactivation is not reversible by dialysis or by reduction with dithiothreitol, and is greatly attenuated in the presence of supernatant dehydroascorbate.

Glutamine synthetase is an enzyme with a binding site for glutamate which is structurally similar to that of p-glutamylcysteine synthetase. This enzyme provides a major mechanism for inactivation of glutamate in the central nervous system. We have found that glutamine synthetase is completely inactivated by incubation with 6-hydroxydopamine. This inactivation has the same time course as that of p-glutamylcysteine synthetase and is also attenuated in the presence of supernatant dehydroascorbate. These findings indicate that oxygen radicals may perpetuate their own toxic effects upon the nervous system by paralyzing their own detoxification and by prolonging cellular exposure to glutamate.

Animal: Nina F. Schor* (SPON: P. Wade) Division of Child Neurology, University of Pittsburgh, Pittsburgh, PA 15213

466.2 DEPRESSION OF THE MONOSYNAPTIC REFLEX IN THE NEONATAL RAT SPINAL CORD BY DIFLUOROPROPIONIC ACETIC ACID AND ITS REVERSAL BY ATROPINE. Shyamal Das Gupta* and Jordan E. Warnick (SPON: N.S. Pilates). Dept. of Pharmacol. & Exptl. Ther., University of Maryland School of Medicine, Baltimore, MD 21201.

Difluoropropionophosphorofluoridate (DFP) is an organophosphorus (OP) compound which is known to have multiple actions. In addition to inhibiting acetylcholinesterase, with the consequent elevation of acetylcholine levels at both central and peripheral cholinergic sites, DFP also blocks the ion channel of the nicotinic acetylcholine receptor (Ruba and Albuquerque, Science 181:853, 1973). Recent work in this laboratory has shown that, at least, an irreversible OP agent, facilitated the monosynaptic reflex at 5/20 m and reduced frequency-dependent depression in isolated neonatal spinal cord in which acetylcholinesterase had previously been inhibited by DFP (Yang & Warnick, Fed. Proc. 43:929, 1984). We now report that DFP depresses the monosynaptic reflex in the neonatal spinal cord, an action which is antagonized by atropine. Spinal cords were removed from 6- to 8-day-old female rats, hemisected, placed in an experimental chamber and superfused with physiological solution at 25°C. A monosynaptic reflex was evoked by stimulating the L4 dorsal root and recording from the corresponding ventral root.

DFP caused a concentration-dependent depression of the monosynaptic reflex. At 100 μM, DFP had no effect when applied for 15 min, but caused 8, 33, 54 and 70% depression of the monosynaptic reflex at 1, 10, 100 and 500 μM, respectively, while enhancing frequency-dependent depression. Recovery from DFP-induced depression was dependent upon the concentration of DFP: for the higher the concentration, the longer the washing period before recovery. For example after exposure to 3 μM DFP, recovery after washing occurred in 15-20 min while at 100 μM, it took about 2 hours. The depression caused by DFP could be completely and quickly (<10 min) antagonized by exposure to atropine (500 μM). A lower concentration of atroipe (30 μM) was equally effective in preventing the depression caused by DFP (10-500 μM). The depression of the monosynaptic reflex by DFP was unrelated to inhibition of acetylcholinesterase and is similar to that caused by the cholinergic agonist carbamylcholine and oxtremorine (Swanson and Warnick, Abs. Soc. Neurosci. 10:417, 1984). The beneficial action of atroipe in DFP-induced depression may be attributed to the protection of a cholinergic receptor or mediated by a reduction of presynaptic stores of bound acetylcholine. DFP's action may therefore result from blockade of a cholinergic receptor.

(Supported by U.S. Army Medical Research and Development Command Contract DAMD17-85-C-6030.)
Whether these changes are manifestations of a direct afferent to the neostriatum remains to be determined.

Changes were observed in the electrophysiology of the neostriatum that were in accord with the altered neurochemistry. Field potentials were recorded in the neostriatum in response to activation of a relatively direct input (electrical stimulation of the somatomotor cortex) and a polysynaptic pathway (electrical stimulation of the forepaw). In all DFP-related field potentials of types of field potentials were reduced in amplitude and were unable to follow repetitive stimulation at rates greater than 20 Hz. These effects were most marked in the multiple evoked by stimulation of the forepaw. In addition, the latter responses showed abnormal rapid habituation.

In conclusion, DFP causes persistent changes in the neurochemistry and electrophysiology of the neostriatum. Whether these changes are manifestations of a direct influence or a reflection of changes in structures afferent to the neostriatum remains to be determined. Supported in part by NIH Grant ES00202; T.C.T. is a trainee on NIH Training Grant GM07377.


Supported in part by NIH grants HL27358 and RR00167.)

666.6 DEGENERATION OF THE MOSSY FIBER ENDINGS IN THE HIPPOCAMPUS OF NEAR-TERM RHESUS FETUSES TREATED PRENATALLY WITH DEXAMETHASONE. H. Uno, E. Dong*, M. J. Engel* and J. W. Kemnitz. Wisconsin Regional Primate Research Center, Dept. of Pathology, School of Medicine, Madison, WI 53715.

Our previous studies revealed marked depletion and hypoplasia of the dendritic spines of CA3 pyramidal neurons and synaptic terminals in the stratum lucidum of CA3 region in dexamethasone-treated 162-day fetuses. The ultrastructure of the dendritic spines of CA3 pyramidal neurons and synaptic terminals in the stratum lucidum of CA3 region in dexamethasone-treated 162-day fetuses is comparable to those seen after in vivo intoxication with soman or paraoxon. Electrophoresis of labeled membranes on 7.5% SDS-PAGE under reducing conditions identified a major 160 kD band which was isolated and labeled with H-DFP in the presence and absence of mipafox and/or paraoxon. Electrophoresis of labeled membranes on 7.5% SDS-PAGE under reducing conditions identified a major 160 kD band at the time of injection and killed 2 weeks later. Neostriatal AChE was inhibited more than 95% at 90 minutes. For neurotransmitter determinations, rats were killed by head-focused microwave irradiation. ACh and catecholamines were determined by HPLC with electrochemical detection. Neostriatal ACh levels were elevated at both times after DFP administration. While neostriatal dopamine (DA) levels were not significantly altered, DOPAC levels and DOPAC/DA ratios were increased at both times after exposure to pinacolyl methylphosphonofluoridate (soman), or hypoxia (95% O2 : 5% O2) treated at 4°C. After fixation, small tissue blocks (1 x 2 x 3 mm) were cut and fixed overnight with a routine EM fixative at 4°C. After fixation, small tissue blocks (1 x 2 x 3 mm) containing the dentate and CA3 region (including the stratum pyramidal and lucidum) were dissected and prepared for EM procedures. In untreated hippocampus of near-term fetuses, a large number of the mossy fiber endings contained numerous synaptosomes, and the synaptosomes were present in the dentate gyrus and CA3 region. The results indicate that soman-induced degenerative changes in the CA3 region of near-term rhinoceros fetuses are comparable to those seen after in vivo intoxication with soman or paraoxon. We are investigating the possibility that NTE is the target for these compounds and that the ability of these compounds to cause organophosphateester oxidized depolarized rhinoceros (OPIEM). We are investigating the possibility that NTE is the target for these compounds and that the ability of these compounds to cause organophosphateester oxidized depolarized rhinoceros (OPIEM).

In conclusion, DFP causes persistent changes in the neurochemistry and electrophysiology of the neostriatum. Field potentials were reduced in amplitude and were unable to follow repetitive stimulation at rates greater than 20 Hz. These effects were most marked in the multiple evoked by stimulation of the forepaw. In addition, the latter responses showed abnormal rapid habituation.

In conclusion, DFP causes persistent changes in the neurochemistry and electrophysiology of the neostriatum. Field potentials were reduced in amplitude and were unable to follow repetitive stimulation at rates greater than 20 Hz. These effects were most marked in the multiple evoked by stimulation of the forepaw. In addition, the latter responses showed abnormal rapid habituation.

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In conclusion, DFP causes persistent changes in the neurochemistry and electrophysiology of the neostriatum. Field potentials were reduced in amplitude and were unable to follow repetitive stimulation at rates greater than 20 Hz. These effects were most marked in the multiple evoked by stimulation of the forepaw. In addition, the latter responses showed abnormal rapid habituation. Dissected and prepared for EM procedures. In untreated hippocampus of near-term fetuses, a large number of the mossy fiber endings contained numerous synaptosomes, and the synaptosomes were present in the dentate gyrus and CA3 region. The results indicate that soman-induced degenerative changes in the CA3 region of near-term rhinoceros fetuses are comparable to those seen after in vivo intoxication with soman or paraoxon. We are investigating the possibility that NTE is the target for these compounds and that the ability of these compounds to cause organophosphateester oxidized depolarized rhinoceros (OPIEM). We are investigating the possibility that NTE is the target for these compounds and that the ability of these compounds to cause organophosphateester oxidized depolarized rhinoceros (OPIEM).
466.7 EFFECT OF CHRONIC CORTICOSTERONE ADMINISTRATION ON THE SEPTO-
HIPPOCAMPAL CHOLINERGIC SYSTEM OF THE RAT. Y. Tashiki, V. R. Glid*
and G. M. Glid*. Dept. of Pharmacology and Laboratory Medicine, Harvard
Medical School, Boston, MA 02115 and Center for Neurosci. & Behav. Res. The Weizmann
Inst. of Science Rehovot, Israel.

Degeneration of the septo-hippocampal cholinergic system is
considered to be critically involved in aging and dementia.
Glucocorticoids have been shown to be capable of accelerating the
degeneration of hippocampal neurons. Since the hippocampus
receives an extensive cholinergic input from the medial septum, it
was of interest to examine the effect of glucocorticoids on this
input. Nine male adult rats were injected i.p. once daily, six
days a week for 3 months with 10 mg of corticosterone in peanut
oil. Controls received vehicle injection. Twenty four hours
following the last injection 3 rats from each group were
anesthetized and perfused for histological studies. The remaining
rats were decapitated and the septum, hippocampus, frontal cortex and
striatum were dissected free. "Choline uptake was not
affected in the hippocampus or the striatum. Corticosterone
caused a 30% increase in newly synthesized "3H-acetylcholine
release in the striatum but not in the hippocampus. Choline
acetyltransferase (CAT) activity was reduced in the striatum (30)
and the septal area (20%). Despite an apparent loss of septo-
hippocampal cholinergic cells being affected in the hippocampus or the striatum, Choline
corticoid of rats, causes a spatio-temporal effect on the septo-
hippocampal system with septal cholinergic cells being affected
prior to their target area. Furthermore, we postulate that a
failure to find a decrease in CAT activity in the hippocampus,
with astrocytes and with postsynaptic membrane fragments of in­
put. Nine adult male rats were injected i.p. once daily, six
groups (A and B). Animals of both groups received an injection (ip) of KA at doses of 5,7.5
or 12.5 mg/kg, respectively. Group A was allowed to survive for 6 months, and
Group B was allowed to survive for 12 months. Each animal was sacrificed by intracardial perfusion.

466.9 KAINIC ACID LESIONS OF THE LATERAL GENICULATE NUCLEUS: ALTERED
COARSE OF AXON TERMINAL DEGENERATION FOLLOWING ENUCLEATION. C. K.
Meshul, W. R. Woodward and L. M. Lund*. V.A. Medical Center and

It has previously been shown that kainic acid (KA) injections in
rat dorsal lateral geniculate nucleus (dLGN) completely destroy
geniculate neurons but does not affect the retina, or a visual
cortex which terminate in or pass through this nucleus (Woodward and
scent dyes or HRP injected into KA lesioned dLGNs showed that the
terminal cells of terminals originating in either the retina or visual
cortex were unaffected. Electron microscopic studies revealed the presence of small, medium and large axon terminals, which ap­
pared intact and filled with synaptic vesicles. Many of these
-terminals appeared to “make synaptic-like contact with other axons,
with astrocytes and with postsynaptic membrane fragments of in­
put. In control animals degenerating retinal terminals showed
characteristic darkening of the cytoplasm and clumping of synaptic vesicles. Two distinct regions were observed in small and medium sized terminals: the first wave occurring by 1 day after
enucleation and the second by 15 days. Large terminal
demonstrated evidence of degeneration between days 3 and 7 post­
enucleation, and no degenerating profiles were observed at day 30.

466.10 EXCITATORY AMINO ACIDS DO NOT CAUSE DEATH OF

Excitatory amino acids (EAAs) have been implicated in neurotrophic deficits in several animal models of neurodegenerative disease. Our hypothesis, that EAAs, such as kainic acid (KA), are not neurotoxic to cultured mammalian retinal ganglion cells (RGCs), has received support from our experiments. In KA-lesioned geniculates the rate of degeneration may reflect a compensatory response in surviving cell terminals.

Recent studies have shown that kainic acid (KA) injections in the rat dorsal lateral geniculate nucleus (dLGN) completely destroy geniculate neurons but does not affect the retina or a visual

terminals. For example, in KA-lesioned geniculates, the rate of
terminal degeneration may be significantly slowed, or ultra-

terminals. For example, in KA-lesioned geniculates, the rate of
terminal degeneration may be significantly slowed, or ultra-
466.11 AN EXCITATORY AMINO ACID ACTIVATES CALPAIN I AND STRUCTURAL PROTEIN DEGRADATION IN HIPPOCAMPUS IN VIVO. J.C. Noszek* and R. Siman, Neurosciences Group, Medical Products Dept., The DuPont Co., Wilmington, DE. 19898.

Mammalian brain contains two calcium-activated proteases, calpain I and calpain II, that are activated, respectively, at low micromolar and high micromolar calcium concentrations. Calpain activation has been hypothesized to be critically involved in structural modification of synapses, and in neuronal degeneration. It has not been demonstrated, however, that physiological stimuli can activate the calpains in vivo. We report here that administration of the excitatory amino acid kainate in vivo causes activation of both calpain I and degeneration of neuronal structural proteins.

Rates were administered kainate (12 mg/kg) intraperitoneally and allowed to survive for 1, 3, or 6 hours. The extent of calpain activation was assessed in dorsal hippocampus, taking advantage of the property of the calpains to undergo autodigestion upon activation. Calpains I and II were separated by SDS-PAGE and detected and quantified by immunoblotting with polyclonal antibodies to the Mr~84kD catalytic subunit of human erythrocyte calpain I. Blots of partially purified rat brain calpain I or rat brain calpain II indicated that the antibodies recognized the catalytic subunits of both proteases (rat brain calpain I Mr~84kD, calpain II Mr~76kD). Kainate induced a time-dependent decrease in calpain I but had little effect on calpain II, with calpain I levels decreasing by 55% of control by 6 hours. Concomitant with the calpain I decrease, kainate stimulated the degradation of brain spectrin and the Mr~200kD neurofilament subunit, quantified by immunoblotting with appropriate antibodies. Spectrin proteolysis was accompanied by a four-fold increase in two lower molecular weight breakdown products; these fragments are of identical size as those produced upon cleavage of purified brain spectrin by purified calpain I. In contrast to the spectrin and neurofilament polypeptides, kainate did not affect gliarial fibrillary acidic protein, an excellent calpain substrate in vitro, and did not alter levels of actin, a poor calpain substrate.

These results indicate that excitatory amino acids can provide sufficient intracellular calcium to activate the high-sensitivity protease calpain I, and that calpain is involved in synapse loss in neocortex, but perhaps not in glia, and leads to degradation of major neuronal structural proteins. The findings support the hypothesis that calpain I activation is an obligatory step in the toxic action of excitatory amino acids. Conceivably, less pronounced stimulation of excitatory amino acid receptors than employed here could act through calpain I to mediate more modest structural changes than those associated with neurotoxicity.

Capsaicin induces neuronal degeneration in midbrain and forebrain structures in neonatally treated rats. T.Y. Dinh and S. Ritter (SPON: F. Young). College of Veterinary Medicine, Washington State University, Pullman, WA 99164-6520.

Capsaicin is a neurotoxin that causes degeneration in brain and spinal cord of both neonatal and adult rats. The prevailing view is that this degeneration is limited to areas of the spinal cord and caudal hindbrain that receive primary afferent input. However, using a novel silver stain for labeling degenerating neurons, we recently found that systemic capsaicin treatment of adult rats causes degeneration in discrete brain loci not traditionally associated with primary sensory innervation. Therefore, chronic stimulation of EAA receptors damages hippocampal and cerebellar structures that include a calcium-dependent step. Furthermore, neurotoxic activation is required for toxicity. Coupled with the recent finding (Noszek et al., this meeting) that EAA neurotoxicity can activate the calcium-dependent thiol-proteases calpain I in vitro, the results suggest that calpain I activation may be necessary for the expression of EAA neurotoxicity.

The evidence that schizophrenia may involve infection by a virus (or viruses) has been indirecd. This evidence includes the phenomenology of schizophrenia (including as it may be mimicked by some viral encephalitides), epidemiological factors (including a predilminence in females, such as in early spring births or a north-south gradient, and occasional clustering of cases), and indirect laboratory evidence (glossis in some neuropathological studies, spinal fluid protein abnormalities, and abnormalities in cell-mediated immunity).

The discovery of the human retroviruses, HTLV-I, HTLV-II and HIV, now also known to affect the CNS, together with the development of new techniques in human virology, made it possible to investigate the role of this class of viruses in the etiology of schizophrenia.

From 20 chronic schizophrenia patients and 10 normal subjects short term tissue cultures of peripheral lymphocytes were established. The cells were grown in the presence of T-cell growth factor, and the culture supernatant was tested for the presence of reverse transcriptase activity every three to six days. Under these conditions reverse transcriptase activity was detected in our cultures from patients or normals. To investigate the possibility that a retrovirus is present, but not activated under these conditions, we treated the cells with 2.5-5 mM 5-Azaacytidine. This agent is known to affect viral gene expression by altering the extent of DNA-methylation. In initial experiments no reverse transcriptase activity could be detected in the 5-Azaacytidine treated cultures. Further studies are necessary however to substantiate this finding.

In conclusion, we found no T-cell associated reverse transcriptase in peripheral lymphocyte cultures of our patients. Therefore, these data do not support an association of a T-cell lymphotropic retrovirus with schizophrenia.

467.2 RECOMBINATION FOLLOWING BAY MIDDLE CEREBRAL ARTERY THROMBOTIC OCCLUSION: HISTOPATHOLOGICAL, IMMUNODYNAMIC AND MICROSURGICAL CONSIDERATIONS. H. Nakamura, T. Fujii, B.D. Marson, P. Bostok, and M.D. Olmberg (Spon: R.G. Clark). Cerebral Vascular Disease Research Center, Departments of Neurology and Anatomy and Cell Biology, University of Miami School of Medicine, Miami, FL 33101.

Human thromboembolic stroke is a complex process in which platelets participate either in the activated form or through the release of vasoactive substances, such as prostacyclin, platelet factor 4 (PF4) and interleukin 1 

After 1 hr, 4°C incubation with neural cultures, TTC Hex A was always greater than that of PLL Hex A and much greater than that of Hex A alone. This was significantly smaller in recirculated compared to nonrecirculated rats. Striatal infarction was common, typically affecting 50-70% of the striatum.

Thus, conjugation of TTC to Hex A effectively and specifically enhances uptake of enzyme by neurons in culture. This should obviate the problem of uptake by CNS neurons after BBR peripheralization. The TTC Hex A may also enhance delivery of enzyme to neurons via TTC-mediated axonal transport and transsynaptic transfer (Schwab, M.E., et al. J. Cell Biol.82:798, 1979) and may also enhance uptake of enzyme by neurons in culture. This should obviate the problem of uptake by CNS neurons after BBR peripheralization.

Immunohistological experiments have shown that the anti-encephalitogenic bovine spinal cord protein (SCP) is a component of the cytoplegia of most if not all, neurons, oligodendrocytes and Schwann cells of the bovine, human and rat nervous systems (MacPherson and Wallace, Acta Neuropathol. 50:25, 1980; MacPherson and Benoist, Acta Neuropathol. 59:191, 1985).

Recently, SCP was discovered in normal bovine and human cerebrospinal fluids. At that time it was hypothesized (1), that the SCP was secreted continuously into the CSF by neurons and oligodendrocytes (2), and that major changes in the metabolism of neurons or oligodendrocytes would be reflected in the levels of SCP in the CSF. In order to test this hypothesis we developed a competitive inhibition enzyme immunoassay (EIA) to measure the SCP in the CSF using a rabbit anti-bovine SCP antisera and highly purified bovine SCP (RSPC). The CSF-SCP levels were measured at varying intervals in normal bovine adults, in 6 to 8 month-old calves, and in adults in which severe experimental allergic encephalitis (EAE) was induced by injecting i.c. 250 mg whole bovine spinal cord emulsified in complete Freund’s adjuvant. The daily concentration of RSPC in the CSF of normal calves did not vary by more than ±2.5 ng/ml. SCP was readily induced in adult cattle and clinical signs of disease were usually apparent 21 days after induction. The CSF-SCP values at the time clinical signs of EAE appeared were 2 to 3 times higher than normal. Moreover, peak SCP concentrations occurred before clinical signs were evident. These results suggest that the metabolism of neurons and/or oligodendroglia is changed in EAE and studies are continuing to ascertain which cell is contributing the excess SCP.

Supported by grants from the Research Committee, Department of Veterinary Pathology, Univer. of Western Ontario, London, Ont. NIG 2W1 and Sch. of Veterinary Med., Univ. of Western Ontario, London, Ont. N6G 2K3.}

467.6 T-CELL GROWTH FACTOR ACTIVITY SECRETED BY A NEW HUMAN GLIOMA CELL LINE D.B. Ruskirk, L.J. Marino, Jr., R. Hannon, R.B. Dvorak, and A.J. Fried, Wistar Institute, Philadelphia, PA 19104, and Rockefeller University, New York, NY 10021

As a part of our ongoing studies of the response of human T-lymphocytes to syngeneic brain tumors, cells from a human glioma were selected for their growth factor activity. The new cell line, designated DBA, is a rapidly growing pleomorphic glioma cell line. Tissue culture supernatants from this line support the growth of interleukin-2 dependent T-cell lines.

Tissue culture supernatants were precipitated with 70% ammonium sulfate, dialyzed against PBS, and filtered through a 0.22 micron filter. The resulting material, concentrated ten-fold over the original supernatant, was tested for T-cell growth factor activity in both the HT-2 cell line and independent IL-2 dependent T-cell lines maintained in our laboratory. The preparation supported the growth of the cell lines at an activity of approximately 200 units/ml, where a unit is the reciprocal of the dilution which supports half maximal titiated thymidine incorporation, making this preparation comparable in activity to our positive control, human IL-2 obtained from Boehringer-Mannheim.

The presence of T-cell growth factor activity does not necessarily imply that the cells are producing IL-2 itself. However, SDS-polyacrylamide gel electrophoresis reveals that the tissue culture supernatants contain a 15k dalton protein which migrates indistinguishably from the 15k dalton protein in the commercial IL-2 preparation.

Previous data from our laboratory demonstrated that DBA cells respond to IL-2 by both proliferation and morphological change. Coupled with our new results, we suggest that interleukin-2 can play an autocrine role in tumor biology than as only a cytotoxic T-cell lymphokine.

467.7 DENDRITIC CHANGE FOLLOWING MINOR AND MODERATE TRAUMATIC BRAIN INJURY, J.T. Pavlisko and D.L. Emb*, Department of Anatomy, Virginia Commonwealth University, Richmond, VA 23298.

Previous studies have demonstrated that diffuse traumatically induced reactive axonal change is a consistent feature of the traumatic event, occurring without direct attendant focal change in either the related dendrites, somatic or vascular elements (J. Neuropathol. Exp. Neurol. 42:225-241, 1983). In the present study, these observations were extended by focusing attention on another response to diffuse reactive change that entails the focal separation of the axon, with degeneration of the distal, detached segment, one can infer that deafferentation-induced responses triggered by such diffuse reactive change are likely to represent the fundamental response at a cellular level of the brain and may be intimately related to cellular changes in the brain following diffuse injury.

Animals were subjected to low to moderate intensity fluid- percussion injury and were followed over a 24 hour to 3 month survival period. Reactive axonal change was systematically assed in the vestibular nuclei, where damaged axons are always observed following injury. Anesthetized cats were subjected to low to moderate intensity fluid- percussion injury and were followed over a 24 to 3 month survival period. Reactive axonal damage was identified through the use antiradgrade labeling of the cell body with a WGA-HRP, with subsequent processing for the LM and TEM visualization of the peroxidase reaction product. TEM was used to assess change in the target vestibular nuclei. In the early posttraumatic period, electron dense and detached terminals were observed in relation to numerous dendrites afferent to the second order perinuclear term, these dendrites revealed various changes including myelin figures, autophagic vacuoles and spine loss. In the second posttraumatic week, some dendrites were totally swollen and varicosite-like. These were laden with autophagic vacuoles, myelin figures and various forms of membrane debris. With survival into the third posttraumatic month, few dendritic abnormalities were seen, thereby suggesting dendritic recovery or reorganization. Collectively, all these dendritic changes occurred while many of the reactive axons were mounting a sustained sprouting response. The results of this investigation, demonstrate that traumatically induced axonal damage triggers a delayed reactive change in their target neurons. The relevance of such change to the sequelae of minor and moderate traumatic brain injury will be considered.

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467.8 INHIBITION OF IN VITRO PROTEIN SYNTHESIS IN RAT CEREBRAL CORTEX FOLLOWING FLUID-PERCUSION BRAIN INJURY, G.A. Gape*, A. Alves*, P.S. Dwan*, M.K. Cheung, M.A. Verity*, and R.P. Becker, UCLA School of Medicine, Neurosurgery, UCLA Medical Center, Los Angeles, CA 90024.

While the events of brain damage in head trauma are diverse, cerebral concussion is a basic underlying phenomenon. Despite this, studies of the biochemical events in concussion are scarce. Such studies would seem of particular interest as, at milder levels of injury, they likely represent the fundamental response at a cellular level of the brain to mechanical trauma. The present study was undertaken in an attempt to elucidate some of these biochemical alterations. It was felt that protein synthesis might be a relatively sensitive indicator of perturbed cellular function whose impairment would have important consequences particularly in the setting of repetitive sublethal injuries requiring intact synthetic capacity for cellular recovery.

In vitro incorporation of radiolabelled amino acid was studied in a postconcussional supernatant (PMS) from Sprague-Dawley rat cerebral cortex thirty minutes after a fluid-percussion brain insult, 1 - 2 am in magnitude. Animals were anesthetized with an oxygen-nitrous oxide-ethane mixture prior to injury. Test animals recovered completely following varying periods of coma, which corresponded with the severity of injury. Cell-free protein synthesis was inhibited up to 80% Poly U-directed synthesis of polyphenylalanine was also inhibited. Sedimentation profile of radiolabel incorporation was similar to that from controls. These findings indicate inhibition at the level of peptide elongation. They also suggest that the inhibition seen in this model from that demonstrated in cerebral ischemia with reperfusion occurs at a similar site of inhibition. These findings suggest inhibition of cell-free protein synthesis which is bypassed in the poly U-directed system, as well as accumulation of monoribosomes. Inhibition in these instances is, thus, thought to lie at the level of initiation. Therefore, the inhibition of brain protein synthesis following fluid-percussion brain injury appears unique to mechanical trauma.

Continuing research aims at further defining the site of action and implications of inhibition of polypeptide elongation in this model.

Monoclonal antibodies have been produced against two antigens (G7 and G9) found in Lewy bodies in dopaminergic neurons of the substantia nigra in patients with Parkinson's disease. The antigens have been characterized by immunoblot after two dimensional gel electrophoresis. The molecular weight of the two antigens is 48 kd and 52 kd. Two-dimensional gel electrophoresis of the antigens show that they are not located in the dopamine cell bodies, or a variety of cytoskeletal proteins. They are found in normal subjects and in patients with progressive supranuclear palsy, a parkinsonian syndrome without Lewy bodies, and do not therefore cause Lewy body formation. Absent from the substantia innominata, they are not structurally essential for Lewy body formation. The identity of the antigens remains unknown.

467.10 RESTRICTION FRAGMENT LENGTH POLYMORPHISM STUDY OF CANINE NARCOLEPSY. H. Motoyama*, T.S. Kilduff, W.C. Dement, B.N. Lea*. R.G. Roberts* (SPON: A. Prochiantz* and T. Govoni). Department of Neurology and Medical Microbiology, Stanford University School of Medicine, Stanford, CA 94305.

Narcolepsy, a disorder characterized by periods of excessive daytime sleepiness and cataplexy, has been reported in humans and dogs. In humans, the disease is believed to be inherited by a polygenic mechanism, while in dogs of Labrador breed, the homozygous parents are carriers of a recessive gene (narc-1). Tor 23, a monoclonal antibody (JNC 43775) which binds the surface of motor neurons in ALS, from their central origins to the periphery, thus demonstrating a potentially important epitope in motor nerve structure and function. This study focuses on whether, in both peripheral nerve bundles and ventral roots, we can observe consistent changes occurring along the surface of motor neurons in ALS, from their central origins to the periphery.

467.11 CANINE HLA TYPE AND NARCOLEPSY: IS THERE AN ASSOCIATION? R. Bean*, T.S. Kilduff, W.C. Dement and C. Grumet* (SPON: M. Ellis). Dept. of Psychiatry and Pathology, Stanford University School of Medicine, Palo Alto, CA. 94305.

Narcolepsy is a neurological disorder characterized by excessive daytime sleepiness, sleep onset REM periods, and cataplexy. Population studies have demonstrated that almost 100% of patients with narcolepsy possess the major histocompatibility complex (MHC) Class II antigen, HLA-DR2 compared to only 22-34% for controls. These findings along with family studies are most consistent with multilocus heterogeneity with minimal linkage disequilibrium between the canarc-1, linkage disequilibrium with a specific MHC Class II allele or allele complex is a strong contributing factor in narcolepsy susceptibility.

The canine narcolepsy model resembles the human disease both behaviorally and pharmacologically. In the selected genetic background provided by several breeds of dogs (Boereman pincercher and Labrador retriever), the disorder appears to be transmitted as a single-gene, autosomal, recessive trait, canarc-1. Analyses of one-way mixed lymphocyte culture patterns of reactivity among homozygosity within the major histocompatibility complex (MHC) is not consistent with homozygous expression in the MHC loci. Preliminary data suggest that the genetic factors for narcolepsy is not consistent with homozygous expression in the MHC loci.


ALS is a neurodegenerative disorder of the motoneuron system. We are examining ALS this year to describe the molecular pathology occurring in the course of the disease. Tor 23 is a monoclonal antibody (JNC 43775) which binds the surface of motor neurons in ALS and ALS (Protein 80342), a protein (abstraction, Stephenson et al., this volume) and human (Muscle & Nerve, in press), thus demonstrating a potentially important epitope in motor nerve structure and function. This study focuses on whether, in ALS, we can observe consistent changes occurring along the surface of motor neurons in ALS, from their central origins to their peripheral terminations.

Tor 23 was localized with immunofluorescence on frozen sections cut from human intercostal muscle and ventral root. In normal tissue, Tor 23 binds the external perimeter of the Schwann cell and the axon. Thus, in a cross-section, one sees an outer and inner ring of stain, or a donut configuration. The closely packed nerve bundles display a uniform deposition of donut stain around all axons, with no stain in the interstitial space. In the ALS tissue, 1) there is a diffuse deposition of stain in the donut structure: on some fibers there is a heavy asymmetric concentration of punctate stain around the exterior sheath in a distinct semi-lunar configuration, 2) the broad interstitial space which is present in ALS nerve bundles is diffusely stained, 3) there are distinct holes of no stain, which may be a loss of individual axons or a loss of immunoreactive material on degranulating axons. These alterations were consistently observed in both peripheral nerve bundles and ventral roots. An examination of the ventral root in longitudinal section from normal tissue reveals Tor 23 binding along the apparent axonal surface with a uniform distribution of stain in the node of Ranvier. In ALS, a greater and a non uniform deposition of stain occurs in the node region. This alteration may describe an incipient demyelinating process, but why this region should accumulate antigenic material is not clear.

We have colocated Tor 23 with anti-neurofilament antibody, as an internal marker for the axon. Nerve bundles from normal cases show a 1:1 correspondence of neurofilament and Tor 23 stain. In ALS, the 1:1 ratio is disturbed in that neurofilament is present but Tor 23 staining is faint or absent. The opposite, neurofilament absent with Tor 23 present, is also observed. This change in staining pattern may represent a selective loss of a surface molecule in degenerating fibers and an accumulation of that molecule in regenerating fibers. Of course, the inverse may also be true. The power of this monoclonal antibody is its potential to home in on the molecule undergoing change.
LOCALIZATION OF 70 KDA HEAT SHOCK PROTEIN INDUCTION IN GERBIL BRAIN AFTER TRANSIENT ISCHEMIA. T. S. Nowak Jr., F. Y. Hassan and H. J. Hindes (Neuroscience Program, Cerebral Vascular Disease Research Center, Department of Neurology, University of Miami School of Medicine, Miami, FL 33101).

We have tested whether small intra-ischemic variations in brain temperature interact with ischemia. To study brain temperature, a thermocouple probe was placed stereotaxically into the lumen of a guide cannula 0.5 mm anterior to 4-vein occlusion. Rectal temperature was maintained between 36-37°C by heating lamp, and striatal temperature prior to ischemia was 37°C in all animals. After 5 min of ischemia, the guided cannula and the rats were left unattended for 24 hrs without sacrifice. The fewest changes were seen except very near the lesion, and microtubules may have bound the residual free calcium, indicating that calcium influx is a major cause of organelle damage.

Intra-ischemic variations in brain temperature had no significant influence on energy metabolite levels measured at the conclusion of ischemia. The histopathological consequences of ischemia, however, were markedly influenced by variations in intra-ischemic brain temperature. In the hippocampus, CA3 neurons were consistently damaged at 36°C but not at 34°C. Within the dorsolateral striatum, ischemic cell damage was present in 100% of the hemispheres at 36°C but only in 50% at 34°C. Ischemic neurons within the central zone of striatum were not observed in any rat at 34°C, but in all rats at 36°C. The extent of neuronal injury at 36°C was controlled, brain temperature fell from 36°C to 30-31°C during the ischemic insult. In this group, no ischemic cell change occurred in the striatal areas and was only inconsistently documented within the CA1 hippocampal region. These results demonstrate that severe depletion of brain energy metabolites during ischemia at all temperatures, small increments of intra-ischemic brain temperature markedly affect the extent of damage. Tissue oxygen tensions did not recover promptly after repetitive cycles of occlusion and recirculation, consistent with diminished capillary perfusion. These results suggest that secondary progressive hypoxia may play a role in the dramatically increased edema and tissue injury following repeated ischemic episodes in gerbil brain.


The effect of repeated ischemic insults on a number of physiological parameters was evaluated in a gerbil model, in which an implanted device allowed repetitive bilateral common carotid artery occlusion of any desired duration and frequency. In particular, we have compared the consequences of three 5 min occlusions from single and 30 min occlusions. All occlusions were done under anesthesia with 2% halothane in 70% N0.15O. Regional cerebral blood flow, determined by [14C]nicotine uptake, regional water content determined as specific gravity; regional vascular volume as capillary distribution space (calculated from blood red cells); and tissue oxygen tension determined polarographically with platinum microelectrodes. The status of brain microcirculation was also evaluated morphometrically by the counting of vascular channels filled with Evans blue under fluororescence microscopy. These data indicate that the blood flow fell to one quarter of control values of approximately 200 ml/100 g/min at undetectable levels during occlusion, and returned briefly to the control range at 2 min. Reciprocally, regional water content showed a characteristic period of hyperperfusion with flow averaging 30-35 ml/100 g/min. A similar pattern was seen in hippocampus and caudate, while blood flow in cerebelum did not change. Following repeated occlusion, peak values at 3 min recirculation were only 60% of control, but hypoperfusion was identical after each occlusion. Significant recovery occurred within 6 hrs recirculation following either single or multiple occlusions, and no reduction in flow was detectable by 24 hrs. Edema after repeated ischemic insults, but also tended toward recovery at 6 hrs. At 24 hrs following repeated occlusions, however, a second phase of marked edema was noted in the regions ischemic with the cumulative effect significantly greater than that observed following a single 15 min occlusion. Total vascular volume was reduced by 40-50% during repetitive ischemic and recirculation. Edema, extending to cortex by 24 hrs but remaining low in thalamus, striatum and hippocampus, was reduced in proportion to brain edema. Tissue oxygen tensions did not recover promptly after successive cycles of occlusion and recirculation, consistent with diminished capillary perfusion. These results suggest that secondary progressive hypoxia may play a role in the dramatically increased edema and tissue injury following repeated ischemic episodes in gerbil brain.
467.17 VERAPAMIL DOES NOT ENHANCE SURVIVAL OF CULTURED MAMMALIAN SPINAL NEURONS AFTER NEURITE TRANSECTION. R.Y. Shi*, J.H. Lucas, and S.H. Gross. (SPOR. P. OUTKIN) Dept. of Biological Sciences, P.O. Box 2106, North Texas State University, Denton, TX 76203.

The ultrastructural deterioration and cell death following a variety of lethal insults including hypoxia and physical trauma have been correlated with an increase in the intracellular concentration of free calcium. Calcium channel blockers have been demonstrated to be protective for reduction of renal cell death after azoxin and for minimization of the area of necrosis after myocardial infarction. The purpose of the present study is to determine the efficacy of calcium antagonists to protect nerve cells after a physical injury of known severity.

Spatially confined 13-14 day embryos were dissociated and grown in monolayer cultures for 3-4 weeks. Matched pair experiments were performed using two cultures from the same seeding date. In each culture a primary retraction was performed from each of ten experimental cells at a lesion distance of 100 μm from the peri-collaterals. Ten other control cells were examined. Cell surgery was performed as previously described using a UV laser microbeam (ibid. Gross et al., J. Neuroscience 3:1979, 1983; Lucas et al., CNS Trauma 2:231, 1985). The tectum itself consists of two domains, a central elongated growth cones, smaller than 5 μm in length enter the optic stalk between 34 and 35 h PF. As the pioneers approach the chiasm some 100 μm proximal to the growth cone, the second group and a front of numerous axons has entered the stalk.

For ongoing investigations on axonal navigation in the visual system of zebrafish, a timetable of axonal growth in the developing retinotectal pathway had to be established. Zebrafish embryos were staged (10 Min) within 1 h post fertilization (PP) and incubated at 28°C. HRP was applied to the developing retinotectal pathway hourly intervals between 30 - 52 h PP. Axons most often were tipped with growth cones and some with terminal arbors in larval tectum resembled those in monolayer cultures for 3-4 t weeks. Primary dendrites were transected in whole mounts of the brain (40 h - 9 d). Tecta were reacted with DAB then sectioned. HRP-applications localized to retinal quadrants, labeled axons were counted and measured. Data from over 150 observations were separated into 3 groups by lesion diameter (1.5-2.3 μm, 2.5-3.3 μm, 3.4-4.5 μm). Within each group the data were further organized based upon the extent of proximal segment transection point (≤ 100 μm, >100 μm).

The percent viability of the cells in each retraction bin showed clearly that, as the lesion diameter increased, the viability of nerve cell survival also increased in a linear fashion (>0% in all cases). For example, within the 2.5-3.3 μm diameter group the probability of survival increased from 0.005% to 96% when retraction was 21-25 μm. We also calculated the retraction/lesion (R/L) probability which improved the probability of survival for cells at each R/L value. The resulting function demonstrated that the probability of nerve cell survival increased linearly with increasing lesion diameter.

Enhancement of the probability of nerve cell survival by retraction of the proximal segment is most likely the result of increased resistance to injury currents (I(inj)), impinging directly on the cell at the lesion. This increased resistance is probably due to more efficient membrane recycling as well as modulation of organelle and subcellular structures at the tip of the severed neurite ("Debré-Ramming"). These data again indicate that at the lesion the probability of neuron survival after physical injury close to the perikaryon.

For Fluoro-Gold labeling, 20 nl of a 42 solution was injected intracranially over the right tectum (2 wk old fish only). Two 4 wk later, eyes were whole-mounted and brains were resectioned and examined with a fluorescent microscope.

The youngest larvae, fibers projected to tectum, most were tipped with growth cones and some with terminal arbors (median size of deep arbors: 35 x 45 μm, mean diameter). We now wish to report that retraction of the proximal segment of a severed optic fiber enhances the probability of nerve cell survival after transection injury.

467.18 SURVIVAL OF CULTURED MAMMALIAN SPINAL NEURONS AFTER NEURITE TRANSECTION INJURY IS ENHANCED BY PROXIMAL SEGMENT RESECTION. J.H. Lucas, J. Cataley, and S.H. Gross, Dept. of Biological Sciences, P.O. Box 2106, North Texas State University, Denton, TX 76203.

We have shown that nerve cell survival after amputation of primary dendrites within 200 μm of neuronal perikarya is a function of the physical parameters of the transection lesions (Lucas et al., CNS Trauma 2:231, 1985). The probability of survival increases with increasing lesion diameter. We now wish to report that retraction of the proximal segment of a severed optic fiber enhances the probability of nerve cell survival after transection injury.

Dissociated mouse spinal cords from 13-14 day embryos were grown in monolayer cultures for 3-4 weeks. Primary dendrites were transected from 10 neurons in a culture while 10 neurons in the same culture served as unoperated control cells. Cell surgery was performed as previously described using a UV laser microbeam (ibid. Gross et al., J. Neuroscience 3:1979, 1983). A primary neurite was amputated at a lesion distance of 300 μm from the edge of the perikaryon of each experimental cell. After lesioning, a single laser shot was used to make a small crack below the surface of the culture substrate at the target point. This mark served as a visible reference for subsequent observations of retraction. Resections of lesion distance and retraction were made from photographs taken just before and 5 min after surgery.

The percent viability of the cells in each retraction bin showed clearly that, as the lesion diameter increased, the viability of nerve cell survival also increased in a linear fashion (>0% in all cases). For example, within the 2.5-3.3 μm diameter group the probability of survival increased from 0.005% to 96% when retraction was 21-25 μm. We also calculated the retraction/lesion (R/L) probability which improved the probability of survival for cells at each R/L value. The resulting function demonstrated that the probability of nerve cell survival increased linearly with increasing lesion diameter.

Enhancement of the probability of nerve cell survival by retraction of the proximal segment is most likely the result of increased resistance to injury currents (I(inj)), impinging directly on the cell at the lesion. This increased resistance is probably due to more efficient membrane recycling as well as modulation of organelle and subcellular structures at the tip of the severed neurite ("Debré-Ramming"). These data again indicate that at the lesion the probability of neuron survival after physical injury close to the perikaryon.

467.18 FORMATION OF THE RETINOECTAL PROJECTION IN LABOR GOLDFISH. P. A. Raymond and C. A. O. Stuermer. Univ. Michigan, Dept. Anat. & Cell Biol., Ann Arbor, MI 48109. The organization and regeneration of the retinoectal projection has been thoroughly investigated in adult goldfish, but little is known about developing eyes. We used anterograde transport of HRP and retrograde transport of Fluoro-Gold labeling in larval goldfish from hatching to 2 wk old.

Two ages of larval goldfish were used: newly hatched to 7 days old and 2 wk old. For HRP labeling, a 1% solution of HRP crystal of HRP was injected into one eye. Optimum transport times were 15 mins (2-day-old fish) and 2 hrs (2-wk-old fish). The eyes were injected with DAB then whole-mounted or embedded in methacrylate and sectioned. For Fluoro-Gold labeling, 0.1% of a 42 solution was injected intracranially over the right tectum (2 wk old fish only). Two 4 wk later, eyes were whole-mounted and brains were resectioned and examined with a fluorescent microscope.

In the youngest larvae, fibers projected to tectum, most were tipped with growth cones and some with terminal arbors (median size of deep arbors: 35 x 45 μm). Median arbors are a fraction of total area was 2.7%. In older larvae, arbors were larger (median: 50 x 100 μm), but the relative coverage was the same (mean: 12% of total area). More arbors in deep SPFG (Fp) were 100 x 150 μm (Stuermer, 1986, J.Compr. Neurol. 229:214), but they occupy only a small fraction (0.15%) of tectal area. The shapes of terminal arbors in larval tectum resembled those in adult goldfish (Y-shape for small, rectangular for restricted and a half moon shaped zone of elongated unidifferentiated cells surrounding the core region, with growth cones closely apposed to the most advanced axons. The lateral view even reveals some of c. 100 μm length, arising over 50 μm of the axon's distal end. Occasionally one of the branches is tipped with a growth cone. By 70, 70% have filled the core region (now 250 x 150 μm) and small to medium size arbors are 35 x 45 μm are discernible. From 70 - 72 h onwards, after HRP-application and retrograde labeling, labeled retinal axons were found in retinotopically corresponding tectal quadrants. The formation of a retinotop map coincides with the time at which zebrafish embryo hatch.
468.4 RETINAL TRANSPLANTS FAIL TO SHOW TARGET-DIRECTED OUTGROWTH IN ANOPTHALMIC MICE, M. H. Hankin and R. D. Leopold, Department of Neurobiology, Anatomy and Cell Science, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261.

We have found that retinal transplants placed deep in the midbrain show a directed outgrowth through the brain parenchyma to the superior colliculus (Brain Res. 408: 344 (1987)). This suggested that cells in the colliculus exert a tropic or tropic, influence on retinal axons. The present studies were directed to address the question of whether a superior colliculus that had not previously received optic input during normal development could still attract retinal axons.

Embryonic (E13) Sprague-Dawley rat retinas dissected into the cerebral aqueduct of normal newborn mice (CD-1) or to a strain containing the recessive gene which results in optic retardation (or1). In the heterogenous condition (or1+) the animals appear normal. Whereas the homozygous (or1or1) is anophthalmic.

After 3 weeks the animals were perfused with 4% paraformaldehyde and their brains cryoprotected with 30% sucrose and sectioned on a freezing microtome at 20 μm. Transplant projections were examined with a normal silver stain or with a degeneration stain in cases where immune rejection of the graft was precipitated by a skin graft placed on the flank of the animal.

Retinas placed in mice in which one eye was removed at birth (CD-1 or or1) exhibited a vigorous and directed outgrowth towards the denervated superior colliculus. This projection was distributed throughout the deepstereoforeted supralar layers of that colliculus. In contrast, little or no outgrowth could be detected from retinas placed in intermutant mice (or1or1), although ganglion cells could be identified in these retinas. In the occasional case where limited outgrowth was seen in the mutant animals, the large fascicular character of the projection into normal animals was not observed and the fibers failed to show a strong orientation towards the superior colliculus.

Although it is possible that the or1 gene defect may directly affect the superior colliculus itself, the present observation suggests that in order for the colliculus to exert a positive influence on optic axons (either tropic or tropic in nature) it must have received prior innervation. This raises the possibility that the first optic axons to reach the tectum may induce the production of a target-derived factor which affects the ingrowth of subsequent axons.

Supported by NIH grants NS07817 (MHIH), EY05283 and EY05308 (RD).
648.7 The Role of the NMDA Receptor in Eye-Specific Segregation in Three-Eyed Tadpoles. B.T. Cline & M. Constantine-Paton
Biology Dept. Yale University, New Haven, CT 06511.

We are using the three-eyed frog to examine the development of segregation of retinal afferent terminals into eye-specific stripes. APV, a specific NMDA receptor antagonist causes stripe desegregation when applied chronically to the tectum [Cline et al (87) PNAS 84:12]. The NMDA receptor/channel conducts calcium only when the receptor binds transmitter while the membrane is simultaneously depolarized by other inputs. We suggest that activation of the NMDA receptor allows the tectal neurons to recognize and maintain co-activating afferent synapses.

Application of NMDA to the tecta of three-eyed tadpoles has the curious effect of producing straighter stripes with sharper borders compared to untreated animals. We counted the number of HRP-labeled processes crossing from stripe to stripe. It was found that the number of HRP-labeled processes crossing from stripe to stripe is much greater in untreated regions of tectum. Exogenous NMDA may desensitize the NMDA receptors so that a higher level of afferent cocactivity is required to trigger synapse stabilization, resulting in sharper borders.

To pursue the response to NMDA and APV, we are developing a tectal slice preparation. Slices are cut on a vibratome and visualized on a fixed stage microscope equipped with a cooled stage. Intracellular recordings indicate that the neurons are healthy, with resting potentials in the range of -50 to -70 mV. Although some neurons are relatively silent, many exhibit continuous electrical activity and can be driven to fire action potentials repeatedly with anode break excitation. We have labeled small populations of neighboring retinal ganglion cells with TRITC. Slices can be cut through the tectum and the diencephalon to include dye-filled axons in the optic tract and their terminal fields in the tectum. This preparation allows electrical stimulation of the retinal inputs while recording from their target tectal neurons. We plan to characterize the tectal neurons according to electrophysiological and pharmacological criteria. This work was supported by NIH grants EY05818 & EY06039.

648.8 Kynurenic Acid Blocks Retinal-Tectal Transmission in Rana Pipiens. E.A. Debski, B.T. Cline & M. Constantine-Paton
Biology Dept. Yale University, New Haven, CT 06511.

It has recently been shown (Cline et al, PNAS, 84:12, 1987) that the A-methyl-D-aspartate (NMDA) receptor antagonist kynurenic acid blocks the eye-specific segregation of afferents in the tecta of three-eyed frogs. This finding suggests that the NMDA receptor, a subclass of glutamate receptors, may play a role in establishing retinal-tectal topography in nonmammalian vertebrates.

We are investigating the frog retinal-tectum system pharmacologically in order to determine where glutamate is used as a neurotransmitter. To do this we have developed a cannulated tadpole preparation in which the circuitry between the tecta and other areas of the brain remains intact and the animal can be recorded from without the use of neuromuscular blockers which are known to affect tectal activity. Furthermore, anaesthetics, to which the preparation is acutely sensitive, is prevented by supplying oxygenated Ringers through a cannula, one into the heart and the other positioned caudal to the tecta themselves. The optic nerve is stimulated using a bipolar electrode and the evoked field potential has a stable shape that can be recorded in the tectum for at least 8 hours. This potential consists of a large positive component followed by a large negative one. Both components are potentiated by repetitive stimulation delivered at a frequency as low as 0.5 Hz. The inclusion of cobalt chloride in the Ringers blocks all of the negative component and most of the positive one, indicating that they reflect activity largely post-synaptic to the afferents. The general glutamate receptor antagonist kynurenic acid, in concentrations ranging from 0.1 to 1.0 μM, reversibly blocks a large proportion of both of these components in a dose-dependent manner. This result suggests that most, if not all, of the retinal ganglion cells may use glutamate as a neurotransmitter. We are currently examining the effect of APV on this preparation.

Supported by NIH EY05818, EY05829 and EY06039.

648.9 Antibody Markers for Axons and Oligodendrocytes in the Developing Frog Brain. E. Psina, J. Tashjian and M. Constantine-Paton
Dept. of Biology, Yale University, New Haven, CT 06511.

We have been examining the binding of two monoclonal antibodies (mabs) raised against Rana pipiens brain in an ongoing effort to study developing retinal processes and tectal cells. One of these mabs (LINC) recognizes most of the axons of the frog's tectum from early tadpole stages to the adult. On immunostaining of detergent extracted frog brain LINC recognizes 2 bands in the 60-63kd range. In the tadpole tectum LINC stains some axons in layer 6, processes in the differentiated neuropil and processes within the mantle layer of caudal tectal regions prior to their invasion by retinal axons.

The second mab (OLIG) stains central and peripheral myelin and the somata and processes of the cells giving rise to these sheaths in the CNS. However it appears to bind only to those oligodendrocytes that are myelinating surrounding axons. Thus in the tadpole optic tract OLIG is never observed among the youngest retinal ganglion cell axons adjacent to the glia limitans although some process and myelin staining is pronounced among the deeper, more mature retinal axons. In the tadpole tectum OLIG staining is present in both retinotopic layer 9 and in tectal efferent layer 7 of the most rostral, mature, tectal regions. This reactivity stops abruptly in a distinct midtectal zone lying rostral to the most caudal limits of tectal differentiation and retinal axon invasion. OLIG staining has never been observed in dissociated tectal cells in culture. LINC and OLIG binding has been examined in the contralateral visual pathway of tadpoles following unilateral optic nerve crush. LINC binding is lost over a period of 1-3 weeks following optic nerve crush through the appearance of bright puncta which may signal degeneration of the optic nerve. OLIG binding persists however. OLIC staining is absent in tectal layer 9 by 11 days post-crush although OLIG binding to somata, processes and myelin, process of the efferent layer 7 is unaltered.

Thus LINC and OLIG are provocative of a cytoskeletal antigen that is relatively slow to degenerate following separation from neuronal soma whereas OLIC reactivity seems dependent on the availability of sufficiently mature metabolically intact axon. Supported by NIMH training grant MH00197 to PS and NIH grant EY00639 to KEP.

648.10 The Influence of Thyroxine on Tectal Cell Proliferation. M. Constantine-Paton & B.T. Cline
Biology Dept. Yale University, New Haven, CT 06511.

The hormone thyroxine is responsible for metamorphosis in the frog R. pipiens. To examine the influence of thyroxine on the development of the retinotectal system, we performed a study using thymidine autoradiography to reveal patterns of cell proliferation in the tecta of tadpoles exposed to thyroxine. In one series of experiments, tadpoles (T & T. st. V/V) were raised in thyroxine (1.25 mg/l) for up to 2 months. Animals were injected with thymidine, sacrificed within 48 hours, and their brains were processed for autoradiography. Short term thyroxine treatment (1-3 weeks) results in the superproliferation of cells in the crescent shaped proliferative zone at the caudal end of the tectum. The caudal proliferative zone includes more thymidine-labeled cells than seen in untreated animals and the density of silver grains per cell is greater than in controls, suggesting that thyroxine may increase the cell cycle time of proliferating cells. Longer exposure to thyroxine (8 weeks) followed by thymidine labeling results in the incorporation of thymidine into two additional populations of cells in the rostral, relatively mature portion of the tectum: one population of cells in rostral layer 6, and a second found in the ventricular layer in sections slightly caudal to the layer 6 proliferative zone. Brains from animals with short (1/2 hr) post-thymidine survival times indicate that the layer 6 cells were generated in layer 6, as opposed to being generated in the ventricular layer and migrating to layer 6. Re-examination of brains from untreated animals reveals a few thymidine-incorporating cells in layer 6 and the ventricular layer. It is commonly thought that cells generated late in development, such as those in layer 6, are exclusively glia. We are using the neuron- or glia-specific monoclonal antibodies, LINC and OLIG, described in the previous abstract (Steen et al) and commercially available antisera, in combination with autoradiography to determine whether the late-dividing layer 6 cells are neurons and/or glia. This work is supported by NIH grants EY05818 & EY06039.
SPARING OF THE VISUAL FIELD IS ASSOCIATED WITH LESS METABOLIC DEPRESSION IN THE SUPERIOR COLICUS OF NEONATAL VS ADULT RATS

D.A. Hovda.

...involves a "switching" mechanism which seems to be located in the thalamo-abdominal ganglia of these crustaceans.

468.12 IPSILATERAL VISUOTECTAL PLASTICITY PERSISTS AFTER LESIONS OF ONE NUCLEUS ISTITHI IN XENOPUS.

S.B. Mbadu. Dept. of Physiology, SUNY, Buffalo, NY 14214.

Visual input during development has a profound affect on binocular maps in the tectum of the frog, *Xenopus*. Input from the ipsilateral eye is relayed to the tectum via the opposite nucleus isthmi (NI); this ipsilateral map normally is in register with the retinotectal map from the contralateral eye. Aberrant visual input due to crossed isthmotectal axons induces the crossed isthmo-tectal axons to change trajectories and establish a new map in register with the retinotectal map. The major cue which aligns the maps is the correlation of visually-evoked activity from the two eyes, but we do not yet know which specific axons and dendrites are involved in these processes.

The goal of this experiment was to determine whether the aberrant activity of the retinotectal axon is sufficient to replace the crossed isthmo-tectal axons or whether the uncrossed isthmo-tectal projection also is necessary. This question was raised by the observation that contralateral input elicited by a visual stimulus reaches the tectum much earlier (about 30% more) than ipsilateral input which arrives via a polysynaptic relay (Fig. 1). However, the input from the contralateral eye is later "echoed" by the reciprocal topographic relay shown in Fig. 2. Could the crossed axons receive their cues from this delayed activity? In order to answer this question, I examined the ipsilateral maps in frogs with unilateral lesions of the NI.

One eye was rotated by 45°-150° in midlarval tadpoles (stages 55-57). At stages 60-61, electrolytic lesions were made in the right NI. At 23-25 weeks after metamorphosis, the ipsilateral and contralateral projections to the right tectum were mapped. The sizes of the lesions were measured in ACR-realted sections; 48-80% of the right NI was ablated in 5 frogs; the NI was essentially intact in 3 others. In 7 of these 8 frogs, most or all of the ipsilateral map was aligned with the contralateral map. The sole exception was the map from the frog with the largest eye rotation (150°). Thus, the crossed isthmo-tectal fibers which relay the ipsilateral input to the tectum require the uncrossed isthmo-tectal axons in order to align with the contralateral retinotectal projection.

**Fig. 1**

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468.13 FUNCTIONAL SWITCHING OF VISUAL CIRCUIT COMPONENTS IN SPLENDRAIN CRAYFISH.

D. Barrera-Mera, M. Najar-Rojo, and F. Vargas-Aragot.

In many cephalopods and vertebrates, the nucleus oolfactoretinalis project to the retina, terminating in the inner plexiform layer. Many of these cells contain an antigen specifically recognized by antibodies to FMRF-amide, a molluscan cardioexcitatory peptide (Stell et al., 1984). The immunocytochemical approach to trace the development of these cells in the rainbow cichlid.

In these fish development occurs very rapidly. The eggs hatch within 48 hours after being laid (when maintained at 8°C). At this stage the nervous system is still immature—retinal layers will not be visible for approximately 12 hours. These for days later the fry begin to feed, and they fall within 12 hours of first swimming.

In adult fish, as reported by Stell et al., cells in the nucleus oolfactoretinalis are very immunoreactive for FMRF-amide and react with the FAP procedure to demonstrate immunoreactive sites.

**Fig. 1**

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468.14 DEVELOPMENT OF THE NUCLEUS OLFACTORETINALIS IN THE RAINBOW CICHLID.

Sharon J. Heyman and Anne C. Rounsef. Department of Biology, Montana State University, Bozeman, MT 59717.

In many cephalopods and vertebrates, the nucleus oolfactoretinalis project to the retina, terminating in the inner plexiform layer. Many of these cells contain an antigen specifically recognized by antibodies to FMRF-amide, a molluscan cardioexcitatory peptide (Stell et al., 1984). We have used an immunocytochemical approach to trace the development of these cells in the rainbow cichlid.

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649.1 TRANSMEMBRANE POTENTIAL OF FUNCTIONALLY IDENTIFIED GROUP I AND II FIBERS PRODUCED BY SEGMENTAL AND DESCENDING INPUTS IN THE CAT SPINAL CORD. 
G. Jonides*, M. Solodkin* & P. Rudomin. (SPON: Machado-Salas, J.P.)

CINVESTAV, Mexico 07000.

In the cat, the spontaneous activity of single afferent terminals anesthetized with nembutal we have recorded the transmembrane potential changes produced by segmental and rubrospinal inputs on 83 medial gastrocnemius (MG) afferent fibers. The fibers were in continuity with the muscle. 29 fibers responded with a burst of discharges during the muscle twitch and were classified as tendon organ fibers (94% of these fibers had conduction velocities above 75 m/s and may be considered Ib). In five fibers sural nerve stimulation with pulses 2.5-20X (produced primary afferent depolarization (PAD) and either facilitated, interacted linearly or occluded the PAD produced by group I volleys to the posterior biceps and semitendinosus (PBS) nerve. In 14 Ib fibers SU stimulation produced no PAD, but when applied to facilitation (in 12) led to a PAD produced by PBSSt stimulation. Only in one tendon organ fiber SU stimulation produced dorsal root reflex like activity (DRRx's). The effects of repetitive stimulation of the red nucleus (RN) were analyzed on 8 Ib fibers. RN stimulation produced PAD in 3 fibers, no transmembrane potential changes in 38 spindle afferents. However, SU stimulation inhibited the PAD produced by PBS group I volleys in 26 fibers and facilitated the PAD in 4 fibers. 86% of the fibers did not show conduction velocities above 75 m/s and were classified as group II. These afferents were located below 70 m/s and it is likely that they belong from group II. In 4 of them SU stimulation produced DRRx's and in 2 SU stimulation produced no PAD but inhibited the PBS induced PAD. In the other fibers SU stimulation had no effect. Stimulation of the RN produced no PAD in six Ib fibers. In three of them RN stimulation had an effect, and in the other two the effect occurred by PBS stimulation, and in one fiber the PAD was facilitated. These results are in agreement with previous findings showing that SU stimulation may either facilitate or inhibit the PAD of group II fibers (J. Neurophysiol. 36:987, 1986). They suggest also that group II fibers have transmembrane potentials produced by interneurons which are rather high than Ib fibers, in contrast with what has been reported by other investigators (J. Neurophysiol. 26,1,1963). Partially supported by grants NIH NS01916 and CONACYT PCEBINA 021785.

649.2 A comparison of homonymous and heteronymous connectivity between la and spindle group II fibers and motoneurons in the spinal cord of the cat. R.-H. Ulfhake and U. Yazdani. (SPON: M. Wiesendanger). Department of Physiology, University of Berne, CH-3012 Berne, Switzerland.

Since the motoneurons (MNs) supplying different muscles (e.g., medial and lateral gastrocnemius) are innervated to a great extent in the motor column of the spinal cord, the afferent fibers from these muscles must be guided in a particular way to establish the connections that mediate their localized stretch reflex. Cell surface anionic determinants have been proposed as a chemical marker for recognition between afferent fibers and MNs. In this study we could demonstrate that the sizes of the MNs and muscle afferent fibers determine their topographic relations to each other play decisive roles in determining connectivity.

Multi-unit spike triggered averaging was used to determine connectivity between la and spindle afferents from the medial gastrocnemius muscle and the MNs innervating the medial and lateral gastrocnemius-soleus muscle. Only possible connections between 24 MNs and 12 afferent fibers were studied in single, acute experiments. The influence of morphological and topographical factors as well as of MN species on functional connectivity were analyzed. The probability that a MN would receive functional connections from a given population of afferent fibers was related to its size and its proximity to the MN entry level of the afferent fibers. The faster the axonal conduction velocity of the MN and the closer its location to the entry zone of the afferent fibers the greater was its receptivity. This relationship was qualitatively similar for homonymous and heteronymous la and spindle group II fibers (73 to 75% vs 79% to 83%, respectively) of the MNs (75% vs 79% to 83% of the MNs). Only 12 of the 24 MNs and none of the 12 afferents (combined) had functional connections with homonymous MNs, 22 MNs vs 7 homonymous MNs. However, homonymous and heteronymous MNs of similar sizes were equally likely to receive functional connections when located at the same cranial-caudal level. Differences in the connectivity of homonymous and heteronymous MNs evidently account largely for the observed overall differences in homonymous and heteronymous connectivity. Species recognition apparently appears to play a very minor role in the formation of connections, at least for the close synergists.

The observed pattern of connectivity suggests that connections are formed without further constraints than those given by the architecture and topographical relations of the neural elements involved. (Supported by the Swiss National Science Foundation No. 3.265-0.85).

649.3 SHORTLY ADAPTING PRESSURE RECEPTOR INPUT TO GAMMA MOTONEURONES REVEALED BY CROSS CORRELATION OF UNIT ACTIVITY IN THE CAT. 
P. H. Illingworth* and N. J. Davey*. (SPON: M.C. Wetzel). Dept, of Physiology, University College London WC1 6BT, England.

The background discharges of gamma motoneurones to the gastrocnemius muscle in the cat exhibit a pronounced degree of sustained, irregular discharge with a prominent dynamic component: the background discharges of gamma motoneurones (PLUS NONOIOUS, NEURALISTIC) OF THE KIN OF THE IPSILATERAL SPINAL CORD (ILLINGWORTH, P. H. AND DAVEY, N. J. Exp. Physiol., 70: 233, 1985). This suggests that the activity of certain mechanoreceptors exerts a strong influence on gamma motoneurones in the decerebrate spinal cat. Single afferents from the sural nerve field of the heel were recorded in the dorsal root entry zone of the afferent fibers the greater was its receptivity. This relationship was qualitatively similar for homonymous and heteronymous la and group II fibers (73 to 75% vs 79% to 83%, respectively) of the MNs (75% vs 79% to 83% of the MNs). Only 12 of the 24 MNs and none of the 12 afferents (combined) had functional connections with homonymous MNs, 22 MNs vs 7 homonymous MNs. However, homonymous and heteronymous MNs of similar sizes were equally likely to receive functional connections when located at the same cranial-caudal level. Differences in the connectivity of homonymous and heteronymous MNs evidently account largely for the observed overall differences in homonymous and heteronymous connectivity. Species recognition apparently appears to play a very minor role in the formation of connections, at least for the close synergists.

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649.4 THREE-DIMENSIONAL DIRECTIONAL SENSITIVITY OF NECK MUSCLE SPINDLE AFFECTENTS TO HEAD ROTATION. 

We have recently observed that type A (fairly linear, low gain and dynamic index), 11 ambiguous (3-D response vectors were determined for 44 spindle endings. We have now expanded the study by adding neck rotation in the yaw plane, making possible determination of the most effective direction of simiosidal stretch (response vector) for each afferent.

Using floating electrodes, we recorded from the left C2 DRG of decerebrate, paralyzed cats during head and neck movements. Input to the ganglion was surgically restricted: afferents were therefore in ventral and lateral periventricular muscles, as well as in sternomandoid and, to a lesser extent, clavatopeutus. spindle afferents were identified by their vigorous excitation intravenous acetylcholine. Three-dimensional (3-D) response vectors were either calculated from the gains in roll, pitch and yaw, or approximated from the 2-D vectors in the roll-pitch and pitch-yaw planes determined by "wobble" stimuli (Schor et al., J. Neurophysiol., 51, 1984). Each afferent's linearity was then studied with 2-D planar stimuli, the most effective vector, at least, as close as possible to its vector, using sinemoids of 0.5-5 deg at 0.2 Hz. Dynamic index was measured from response to similarly oriented trapezoidal stretch.

3-D response vectors were determined for 44 spindle endings. Some of the right, roll right ear down, or nose-up pitch were effective stimulating directions for most afferents. Few were dominated by one direction of stretch; instead at least two directions (i.e. pitch and yaw responses). As in the earlier study we observed afferents in three response categories: 21 type A (non-linear, high gain and dynamic index), 12 type B (fairly linear, high gain and dynamic index, Intermediate properties). The coefficient of variation of spontaneous firing rate of type A spindle afferents (CV; 10), while CV values of type B afferents covered a wide range. Five endings had shown type B behavior in response to roll-pitch stimulation, but their response became more dynamic during pitch-yaw stimulation. It seems likely that the remaining 12 type B receptors include secondary muscle spindle endings; type A receptors probably include primary spindles endings (NS02619), (NS17808), NASA (NSG2380) DFG fellowship KA694/1-1 (J.K.), and NIH fellowship NS08050 (B.J.Y.).

The deep muscles surrounding the vertebrae have a rich content of muscle spindles (Bakker and Richmond, J. Neurophysiol. 48, 1982). Recent studies have suggested that these perivertebral spindles provide part of the afferent input to the tonic neck reflex (Chan et al., J. Neurophysiol. 57, 1987). It has also been proposed that medially located propriospinal neurons in C3 form part of the circuitry of this reflex. Propriospinal neurons located in this region, many of which have primary-like and secondary-like properties, are transported transynaptically, thus only the first order efferent projections to segments containing forspinal motoneurons, are modulated by neck rotation (Brink et al., J. Neurophysiol. 54, 1985). It is not known how inputs from neck muscle spindles, which enter the spinal cord through the upper cervical dorsal roots, are relayed to neurons in C4 and C5. We have recently attempted to test the hypothesis that primary neck muscle spindle afferents themselves may carry some of the inputs to the propriospinal neurons.

We recorded extracellularly from spontaneously active neurons in the C2 dorsal root ganglion in decerebrate, paralyzed cats using fine wire electrodes. Afferent input to the ganglion was reduced by denervation of the neck skin and most of the large dorsal root ganglion cells in the C3 dorsal columns with primary-like and secondary-like properties. In addition, the afferents that were classified as spindle afferents were not in the locations where medium sized motoneurons innervate the neck muscles. The afferent fibers were recorded from the propria spinalis of the spinal cord. The preparations of the cells were studied using previously described procedures (Chan et al., J. Neurophysiol. 57, 1987; preceding abstract). An attempt was made to antidromically drive the afferents using a movable ball electrode placed on the dorsal columns. If antidromic stimulation at mid C3 was unsuccessful, the electrode was moved caudally and rostrally. This continued until the afferent could no longer be driven. 23/50 of the tested afferents could be driven antidromically from C3 on motoric levels; 9 could be driven as far caudally as C3, 9 could be driven from C4 but no further caudally, and 7 could be driven from C3. The conduction velocities of these afferents were between 20 and 30 m/sec; the mean ± S.E.M. was 26.7 ± 2.7 m/sec. Latencies to C4 ranged from 0.7 to 1.4 msec. Both afferents with primary-like and secondary-like properties continued until the afferent could no longer be driven. 23/50 of the tested afferents with long descending branches continued until the afferent could no longer be driven. 23/50 of the tested afferents with long descending branches could be best modulated by a wide range of directions of neck rotation.

These results show that C2 dorsal neck muscle spindle afferents project to more caudal segments containing propriospinal motoneurons believed to relay neck muscle activity to forspinal motoneurons. Supported by grants from NIH (NS02619) and NASA (NS23880). B.J.T. was supported by NIH fellowship NS08500 and J.E. was supported by DFG fellowship K456/1-1.


Our recent anatomical findings have determined a significant input to the cat and rat locus coeruleus (LC) arising mostly from the ipsilateral Deiters’ neurons. As the latter cells also contribute inputs descending largely bilaterally (Brink et al., J. Neurophysiol. 54, 1985). It is not known how inputs from neck muscle spindles, which enter the spinal cord through the upper cervical dorsal roots, are relayed to neurons in C4 and C5. We have recently attempted to test the hypothesis that primary neck muscle spindle afferents themselves may carry some of the inputs to the propriospinal neurons. After 24 hr, the animal was reanesthetized and a single injection of 4% Fluoro-Gold (Fluoro-Gold, 4C) was achieved via a microelectrode aimed stereotactically at the lateral LC (1.1 mm lateral to the sagittal suture, 1.2 mm caudal to the lambda, and 5.5 mm below the brain surface). Each animal was then perfused after 24 hr, with warm saline, cold 3.0% paraformaldehyde in 0.1M phosphate buffer, and 30% sucrose solution. The brains and spinal cords were cut in the C3 dorsal root ganglion in decerebrate, paralyzed cats using a cryostat at a thickness of 30 µm. Tissue sections were reacted with TM and labeled with HRP. Fluoro-Gold labeling was screened under the fluorescence microscope by using ultraviolet excitation.

The present analysis included for spinal and brainstem fields containing substantial deposits of Fluoro-Gold at the LC area with minimal spread to the adjacent dorsolateral pontine structures. From these animals, double-labeled neurons were identified in the LC on central processing of vestibular impulses. Supported by grants from NIH (NS24388).
469.9 DIFFERENCES IN THE MORPHOLOGY AND FREQUENCY OF AXON TERMINALS ON PROXIMAL AND DISTAL DENDRITES OF NECK MOTONEURONES IN THE CAT. P.K. Rose and M. Neubert*, Department of Physiology, Queen's University, Kingston, Ont., Canada K7L 3N6.

It was well established that most of the surface area of spinal motoneurones which is available for synaptic contact lies on the dendritic tree. This large area is arranged in an organized fashion which may provide a substrate for segregation of inputs from different sources. In the present experiments, the morphology and frequency of axon terminals contacting different regions of neck motoneurones were compared. Motoneurones were identified electrophysiologically and stained intracellularly with HRP. The position of each axon terminal was determined by matching the relative position of dendrites seen at the electron microscopic level with drawings of the dendritic tree constructed at the light microscopic level.

The axon terminals on two motoneurons innervating biventer cervicis have been examined in detail. All axon terminals of one of these motoneurons were located on dendrites caudal to the soma at least 540 u m from the soma. Axon terminals on the soma and proximal dendrites (up to 240 u m from the cell body) were studied in the other cell. The percentage of postsynaptic membrane in contact with axon terminals and estimates of synaptic density for the somatic, proximal dendritic, and distal dendritic regions are summarized below.

<table>
<thead>
<tr>
<th>Axon Terminal Density</th>
<th>Perimeter in contact with axon terminals</th>
<th>Axon terminal density (number/100 u m²)</th>
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<tr>
<td>Proximal Dendrites</td>
<td>11.9</td>
<td>10.7</td>
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<tr>
<td>Distal Dendrites</td>
<td>22.8</td>
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These differences were associated with differences in the morphology of axon terminals terminating on different regions. 72% of axon terminals on the soma and 57% of axon terminals on proximal dendrites contained either synaptic or pleomorphic vesicles which were tightly packed in clumps. In contrast, these vesicles were rare (8%) on distal dendrites. The most common type of axon terminal on distal dendrites contained a dense collection of evenly distributed spherical vesicles. This type of axon terminal was rarely observed on proximal dendrites (6%) and the soma (1%).

The differences in axon terminal density and morphology may be attributed to functional differences between the two cells or they may be related to the zone of the cell on which the axon terminals were found (Supported by NRC).

469.10 POST-ΤΕΣΤΙΚΑ ΠΟΤΕΝΤΙΑΤΙΟΝ ΤΗΣ ΡΕCURRENT IPSPs IN CAT MOTONEURONES. M.D. Binder and A.D. Lindsay*, Dept. of Physiology & Biophysics, Univ. of Washington, Seattle WA 98195.

During the course of our analysis of the distribution of steady-state, recurrent IPSPs within the cat triceps surae motoneurone pools, we noticed a marked potentiation of the amplitudes of the maximal recurrent IPSPs produced by single shocks delivered to synergistic muscle nerves in response to a single injection of post-tetanic potentiation (PTP) in the la afferent-motoneuron synapse (Luscher et al., Nature 282, 859, 1979 and J. Neurophysiol. 49:269, 1983). In the present experiments, we examined the PTP of recurrent IPSPs in a systematic manner. Of particular interest was the question of whether PTP in this system might be differentially distributed within a pool of motoneurones as is the case for PTP in the la afferent-motoneuron synapse.

Intracellular recordings were made from triceps surae motoneurones of barbilaria-anesthetized cats in which the ipsilateral dorsal roots had been sectioned. The motoneurones identified by antidromic invasion and measurements were made of their input resistances and rheobases. Recurrent IPSPs were evoked by single, supra-maximal electrical shocks delivered to the synergist muscle nerves. Control IPSPs were recorded at a stimulation frequency of 1 Hz. Subsequently, the synergist muscle nerves were stimulated at 500 Hz for 1 sec and the potentiated response was measured 1 sec after the end of the tetanization. Thus far, clear evidence of potentiation has been observed in each motoneuron studied. We will express results with respect to the control IPSPs, the extent of potentiation ranged from 12% to nearly 100%. As expected, the amplitudes of both the control and potentiated recurrent IPSPs were correlated to motoneuron input resistance. Moreover, as is the case for la afferent-motoneuron synapses (Luscher et al., Nature 282, 859, 1979), the degree of potentiation was greater in medial gastrocnemius motoneurones with low input resistances than in more distal those with high input resistances. This difference may be due to the recruitment of motoneurones innervating skeletal muscles and a variant of the standard voltage clamp, that provides these missing data under steady-state conditions (Heckman and Binder, Neurosci. Abstr. 11:402, 1985; Heckman et al., Neurosci. Abstr. 12:247, 1986). In our most recent series of experiments, we have redefined our protocol to 3 nA of current were injected into the cell through the recording microelectrode for 500 ms before and 500 ms during the steady-state IPSP. This procedure provided measurements of both the input resistance (Ri) of the motoneurones (including any significant changes in the membrane's excitability) and the amplitude of synaptic conductances, the synaptic conductance and the amplitude of the steady-state recurrent IPSP. We repeated this procedure using three different levels of injected current so that we could determine the relationship between the amplitudes of the steady-state recurrent IPSPs and the input resistance of the motoneurones. We will characterize, regardless of species, as ZIP (F) or slow (S) as described previously (Engel et al., J. Neurophysiol. 53:1323, 1985).

Discharge properties were examined with miniature haversine depolarizing currents with and without the addition of noise by increasing the number of spikes, peak and average frequencies over 90 s of current injection. Results indicate that type S motoneurones respond to the addition of noise by increasing the number of spikes whereas type F motoneurones do not. With regard to the change in the number of spikes over time of the haversine IPSPs, type F motoneurones accommodated to a greater degree than type S motoneurones. Supported by USPHS grants NS 07888 and HL 07249.


Systematic variation in the amplitudes of synaptic potentials is thought to be the major mechanism underlying the orderly recruitment of skeletal muscles. However, the extent to which this variation can be attributed to differences in effectiveness of synaptic inputs that determine the input resistance of the motoneurones or in the factors that determine the effective synaptic current reaching their somata remains unresolved. Here, we will present data which suggest that the input resistance and the amplitudes of the synaptic potentials produced by several different input systems have generally revealed that these parameters covary, but differences based on this covariance have been limited by the absence of measurements of the actual input, i.e. the effective synaptic current. We have previously described a simple current injection technique, a variant of the standard voltage clamp, that provides these missing data under steady-state conditions (Heckman and Binder, Neurosci. Abstr. 11:402, 1985; Heckman et al., Neurosci. Abstr. 12:247, 1986). In our most recent series of experiments, we have redefined our protocol to the examination of the distribution of recurrent IPSPs and their underlying effective synaptic currents in the cat triceps surae motoneurone pools. Steady-state recurrent IPSPs were produced by applying supra-maximal electrical stimuli to the synergist muscle nerves at 100 Hz (ipsilateral dorsal roots cut) for 1 sec. Small amounts (1 to 3 nA) of current were injected into the cell through the recording microelectrode for 500 ms before and 500 ms during the steady-state IPSP. This procedure provided measurements of both the input resistance (Ri) of the motoneurones (including any significant changes in the membrane's excitability due to the haversine activation of synaptic conductances) and the amplitude of the steady-state recurrent IPSP. We repeated this procedure using three different levels of injected current so that we could determine the relationship between the amplitudes of the steady-state recurrent IPSPs and the input resistance of the motoneurones. The effective synaptic current could then simply be calculated from Ohm's law. We have found that the amplitudes of the steady-state recurrent IPSPs covary with Ri. Moreover, as is the case for the la afferent input system, the underlying effective synaptic currents generated by Renshaw cells appear to be greater in triceps surae motoneurones with high input resistances than in those with low input resistances. Supported by NIH grant NS 22417.


The synaptic noise recorded intracellularly in motoneurones is conditional on the type of anesthesia used during experimentation, with pentobarbital usually reducing the membrane's excitability the most. To determine the effects of membrane excitability on motoneurone discharge characteristics under pentobarbital anesthesia we also repeated experiments to haversine depolarizing currents with and without the addition of pink noise. Surgical preparation consisted of a lumbar laminectomy and a complete left hip and hindlimb denervation. Our intracellular recording techniques have been described previously (Watt et al., J. Neurophysiol. 39:1375, 1976). Haversine current was injected repetitively at 3.85 Hz for at least 90 s at current levels for a single discharge (threshold(T)) and 2T. In addition, at both current levels, discharge properties were examined with and without pink noise (+/-1-3 mA membrane shift, peak to peak). Motoneurones were characterized, regardless of species, as fast (F) or slow (S) as described previously (Zengel et al., J. Neurophysiol. 53:1323, 1985). Discharge properties were analyzed for minimum spike interval, burst duration, time to first spike, number of spikes, peak and average frequencies over 90 s of current injection. Results indicate that type S motoneurones respond to the addition of noise by increasing the number of spikes whereas type F motoneurones do not. With regard to the change in the number of spikes over time of the haversine IPSPs, type F motoneurones accommodated to a greater degree than type S motoneurones. Supported by USPHS grants NS 07888 and HL 07249.

Traditionally, current injection using a rectangular waveform has been used to study the discharge characteristics of motoneurons (e.g. Kehnle, Acta. Physiol. Scand., 66:65, 1965). Recent evidence suggests that other waveforms best resemble the net depolarizing pressure imposed on motoneurons (Hoffer et al., J. Neurophysiol., 57:520, 1987). Preliminary to a neuromuscular-fatigue study on the adaptive properties of motoneurons to repetitive current injections, we considered it necessary to determine whether discharge characteristics of rectangular- and haversine-shaped currents, the latter approximating the locomotor command. Surgical preparation consisted of a dural incision and complete left side hindlimb denervation, all under pentobarbital anesthesia. Our intracellular recording techniques have been described previously (Watt et al., J. Neurophysiol., 39:1375, 1976). Each waveform test was divided into two categories based on pulse duration and current amplitude, yielding 1000 trials (0.50 and 0.25 ms, respectively) representing different types of depolarizing pressure. In 3 cats, 26 transsacral motoneurons were characterized regardless of species as fast (f) or slow (s) as described previously (Zengel et al., J. Neurophysiol., 53:1233, 1985). Discharge properties were analyzed for stimulus spike interval, burst duration, time to first spike, number of spikes, peak and average frequencies at current levels for a single discharge (threshold (T)), 1.5T, 2T and 3T. The analysis to date suggests that there are no differences in the measured parameters between waveform types, for type f and s motoneurons. However, the temporal pattern of spike generation, which is important for the optimization of muscle-force development (Zajac and Young, J. Neurophysiol., 43:1206, 1980) is still being analyzed. Supported by USPHS grants NS 07886 and HL 07249.

469.14 RETROGRADE LABELING OF MOTORNEURONS INNERVATING MUSCULATURE ASSOCIATED WITH THE FOAM GLAND IN MALE JAPANESE QUAIL. C.M. Schacter and P. Oakberg. Psychology Department, Cornell University, Ithaca, NY 14853.

The dorsal prococcal gland (foam gland) of Japanese quail (Coturnix c. japonica) is a sexually dimorphic structure located in the dorsal cloaca immediately posterior to the vent and directly beneath the large circular sphincter ani muscle. The gland secretes a fluid which is injected into a small valvular pocket, produced by the undulation of the sphincter ani muscle (Klemm, R.D. et al., J. Morph., 141:171-184, 1973). Although it is unknown whether the fluid of the foam is transferred to the female during copulation and is also deposited during defecation. The purpose of this study was to locate and characterize the motoneurons responsible for the innervation of the sphincter ani muscle. Horseradish peroxidase (HRP, 8.5 mg/ml) was injected into the target muscle of adult male Japanese quail (mean average body weight 162 gm). Subjects were sacrificed and perfused within 4 hours. Spinal cords and sacrocaudal segments of skin were collected, embedded, and cut into 200 to 250 micrometer sections. Labeled cells were visualized with a modification of the tetramethyl-benzidine technique and counterstained with neutral red. Examination of cloacal musculature confirmed that HRP was restricted to the sphincter ani. Labeled motoneurons with multiple primary dendrites were observed in the 9th and 10th sacral segment of the spinal cord, in the region of the rhomboid fossa. Labeled cells were confined to a nucleus located in Area IX of the lateral horn. Axons exiting through the ventral horn were visible on many of the labeled cells. Japanese quail are a warm-blooded pouched birds in possession of an active foam gland, though smaller, less active glands are present in other species of quail (Schmidt, W.M. and Shalter, M.D., Isis, 114:558, 1972). Although the foam gland and sphincter ani are present in both sexes, the gland and muscle are small in the female in comparison to their counterparts in the male (Klemm, R.D. et al., J. Morph., 141:171-184, 1973). Foam production, gland size, and muscle size are androgen-dependent (Nagra et al., Anat. Embryol., 133:415, 1959) and are positively correlated with strutting and cowering, two sexually dimorphic behaviors (Adkins, E. and Adler, N.T., J. Comp. Physiol., 114:558, 1972). Differences in peripheral structure may be reflected in differences in the central nervous system (CNS), allowing an analysis of the development of such differences may lead to investigations of the development of sex differences in CNS organization, thus providing a more realistic evaluation of sex differences in brain organization and evolution supported by NSF BNS 8412083.


While much is known about the morphology of motoneurons innervating appendicular musculature, little is known about the dendritic organization of motoneurons projecting to axial musculature. We have described the projection patterns of cutaneous and muscle primary afferent fibers emanating from the tail. We are extending our studies of the sacrocaudal spinal cord to include motoneurons by using intracellular injection of horseradish peroxidase (HRP).

Adult cats were anesthetized with Nembutal and the sacrocaudal spinal cord exposed. L5, L4 and/or S2 ventral roots were placed on stimulating electrodes. Motoneurons were identified by a short, antidromic conduction latency. An intracellular electrode position was ascertained by a d.c. shift in membrane potential, by the presence of depolarizing and hyperpolarizing spikes and by the presence of the IS/SD break on the action potential. HRP was injected with positive current for a total of 60 to 120 ma-min and revealed by Banker-Tate's histochemistry.

Preliminary observations, based on intracellular injections of 21 antidromically-identified motoneurons, suggest that: 1) the average diameter of the cell bodies ranged in size from 35 to 65 micrometers (mean of 46); 2) there were numerous dendrites; 3) dendrites were observed throughout the ipsilateral ventral horn and occasionally extended into the base of the ipsilateral dorsal horn; 4) every motoneuron had dendrites reaching into the ipsilateral ventral, lateral and dorsolateral white matter; 5) dendrites reached into the ventral, or occasionally dorsal, to the central canal and projected into the contralateral ventral horn, in some cases lateral ventral and lateral white matter; 5) dendrites extended up to 2300 micrometers rostrocaudally, 1500 micrometers dorsosventrally and 1950 micrometers mediolaterally.

These observations suggest that sacrocaudal motoneurons that project to the cauda equinae are smaller than those of the lumbar cord; have dendrites with considerable spread ipsilaterally, throughout the ventral gray and white matter; have dendrites which usually reach into the contralateral ventral gray and white matter.

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469.16 MICROELECTRODE STUDIES OF LARYNGEAL MOTONEURONS IN THE NUCLEUS AMBIGUUS OF THE ANE VOCALIZING MONKEY. David L. Zealear and Charles R. Larsson. Department of Otolaryngology, Vanderbilt University Medical Center, Nashville, Tennessee 37232 and Department of Speech and Language Pathology, Northwestern University, Evanston, Illinois 60013.

The somatosensory organization and functional roles of laryngeal motoneurons in respiration, and vocalization were studied in the awake fasicularis monkey. Animals were first trained to produce two types of calls for each of the following: a "caw" and a "bark". Following sterile implant surgery, physiological sessions were conducted on each animal. The nucleus ambiguus was targeted using stereotactic coordinates and located with a microelectrode by evoking an antidromic field potential within the nucleus. Isolated recordings from neurons were studied with respect to their firing pattern with behavior. Laryngeal motoneurons were identified using standard criteria, including antidromic invasion, frequency-folow collision. A motoneuron was microstimulated while recording EMG responses to determine the muscle it innervated.

The characteristics of approximately 50 well isolated neurons located within this region of the midbrain representation of the nucleus (i.e., middle third) were examined in detail. Three types of neurons were observed. The most numerous was a nonrespiratory type of motoneuron, which was recruited phasically for both swallowing and vocalization. A second respiratory type of motoneuron was also identified. It was recruited tonically for expiration during both swallowing and vocalization. The third type, found abundantly, was an inspiratory interneuron, which presumably inhibited motoneurons during inspiration. The finding of two types of motoneurons is consistent with previous observations of the various types of motor units (FR, S) and muscle fibers (IIA, I) comprising the muscle. The greater abundance of phasic motoneurons well with the larger proportion of FR motor units and type IIA muscle fibers within the muscle and implicates the muscle in rather ballistic laryngeal functions such as airway protection and pitch nodulation.
469.17 NON-LINEAR INTERACTION BETWEEN BACKGROUND SYNAPTIC NOISE AND 1a SINGLE FIBER EPSPS EVOKED IN SPINAL MOTORNEURONS. M. Boldyrev, D. Ruiz de Leon, L. Jimenez, E. Jimenez. University of Berne, CH-3012 Berne, Switzerland. The mean EPSP produced in the other (test) motoneuron was assigned into one of several groups according to amplitude. The individual EPSPs produced in simultaneously impaled pairs of motoneurons (n=9) were studied in newborn anesthetized cats using spike triggered averaging techniques. In all but one motoneuron pair there was no increase in the coefficient of correlation above baseline levels at the time of the EPSPs. This could imply that the 1a EPSP fluctuations were not correlated, or else, that uncorrelated background synaptic noise precluded detection of correlated 1a EPSP fluctuations. Further analysis was carried out averaging both EPSPs over all trials to permit cursor positioning. The amplitude of each EPSP produced in single trials in one (reference) motoneuron was measured using the predetermined cursor positions and assigned into one of several groups according to amplitude. The mean EPSP produced in the other (test) motoneuron was determined for trials in which EPSP in the reference was in each range of amplitude. In some cases the largest mean EPSP in the test occurred in the trials associated with the largest EPSPs in the reference, and smallest EPSPs in both also occurred in the same trials, etc. In other pairs of motoneurons there were no changes of EPSP amplitude in the test in association with differences in EPSP amplitude in the reference, indicating no correlated fluctuations of EPSPs in the motoneurons. In cases where parallel changes were observed we tested whether the correlated fluctuations were due to parallel changes in synaptic noise that would add to the 1a EPSP under study, thereby resulting in apparently correlated EPSP amplitudes in the motoneuron pair. This evaluation was carried out by moving the cursors in advance of the EPSPs to measure the amplitude of the baseline synaptic noise in single sweeps. In some pairs it was found that the baseline synaptic noise covered the reference and test motoneuron with a slope of EPSP amplitude (i.e. EPSP amplitude) that was 0.01. In similar analysis of 1a-EPSPs we found that the distribution extracted under low noise was not an acceptable solution under high noise conditions suggesting non-linear interaction between high noise and Ia-EPSPs. In simulations in which noise was added linearly to the EPSPs (via computer techniques described above) to achieve baseline variance characteristic of the physiological high noise condition confirmed that the low noise solution (i.e. EPSP amplitude distribution) could apply during high noise conditions. These observations suggest that non-linear interactions may occur between spontaneous and Ia-evoked EPSPs particularly under conditions of increased baseline synaptic noise.

469.18 MORPHOLOGY OF PRIMARY AFFERENT FIBERS AND VENTRAL HORN CELLS IN SPINAL CORD AND BRAINSTEM IV. M. Boldyrev, D. Ruiz de Leon, L. Jimenez. University of Berne, CH-3012 Berne, Switzerland. The organotypic structure of the spinal cord culture with some of its input and output systems preserved together with the good accessibility to the culture was adopted as a convenient model to study the structural and functional interrelations of central and peripheral synapses, as well as the regeneration and function of skeletal muscle. (Supported by the Swiss National Science Foundation No. 3.265-0.85)

Arrays of platinum (faradaic) and anodized, sintered tantalum pentoxide (capacitive) electrodes were implanted bilaterally in the subdural space of the parietal cortex of the cat. Two weeks after implantation both types of electrodes were pulsed with identical waveform consisting of charge-balanced, symmetric, anodic-first pulse pair, 400 μsec/pulse, with charge/ph of 800-1000 μC. 80-100 μC/cm² for 7 hours. Tissues beneath both types of electrodes were damaged but damage was slightly greater beneath platinum electrodes. At the ultrastructural level, in animals killed immediately after stimulation, healthy neurons were intermixed with neurons showing early intracellular edema. Glial cells appeared essentially normal. Animals killed 1 week after stimulation most of the damaged neurons had recovered but the presence of shrunken, vaculated and degenerating neurons showed that some of the cells were damaged irreversibly. We conclude that most of the neural damage from stimulations of the brain surface derives from processes associated with passage of the stimulus current through tissue rather than electrochemical reactions associated with current injection across the electrode-tissue interface, since such reactions only occur with the faradaic electrodes (Supported by Contract HD01-M-62397, Fundamental Neuroscience Program, National Institute of Health).

470.2 Reversal of Increased Muscle Fatigue in Paraplegic Patients by Electrical Stimulation. G. Vrbova*, M.M. Dimitrijevic*, M. Paryi*, J. Halter, Leo Verhagen Metman* Section of Restoration and Rehabilitation, Baylor Coll. Med., Houston, TX, 77030.

It is now well established that activity is an important factor in determining the properties of skeletal muscle fibers such as their ability to withstand fatigue. Increased activity induced by chronic electric stimulation results in a decrease in the fatigability of skeletal muscle in animal experiments (Pette and Vrbova, Muscle and Nerve, Vol. 8, pp. 9-9, 1985) as well as in humans (Vrbova, Vrbova, Hyde and Dubowitz, J. Neurol Neurosurg. Psychiatr. 48: 774-787, 1985). In view of this we tested whether the muscles of patients that have been inactive due to upper motor neuron paralysis for long periods of time would become fatigable, and whether the increased fatigability could be reversed by chronic electrical stimulation.

The fatigability of the muscle was assessed by recording the decrease of force developed in response to repeated stimulation of the tibialis anterior muscle at its motor point with a constant train of stimuli at 40 Hz for 250 milliseconds of each second for three minutes. In control subjects the force of the tibialis anterior decreased by about 35% at the end of three minutes. In paraplegic patients with no voluntary control of both legs the muscles of both legs were stimulated for three to four hours a day. Three months later, when their fatigability was again assessed, their muscles had become significantly more fatigable resistant. Thus electrical stimulation can replace voluntary activity in maintaining fatigue resistance of paralyzed muscles.


Neuroprostheses have been developed for restoration of hand grasp in quadriplegics and gait in paraplegics. The design of feedback control systems for these applications has also been investigated, but the design has been carried out heuristically due to a lack of adequate dynamic models describing the control of stimulated muscle and the biomechanics. We have implemented a computer simulation of the feedback control of a single joint by stimulation of antagonist muscles. Feedback control of joint position and stiffness are being investigated.

A flexible simulating program was implemented. The following elements define the control system: a flexor and extensor muscle; separate internal and external loads; a feedback controller; and a map relating the controller output to the stimuli applied to the two muscles. Each muscle is modeled independently. Muscle force is calculated as the product of the isometric force produced by the stimulation and two terms describing the force-velocity and length-tension properties respectively. The internal load is modeled to represent the passive torque-angle properties at the joint. The external load represents mechanical objects in the environment that make contact with the limb. Both loads are modeled as general second order linear systems. A discrete-time control system was implemented, with a choice of feedback parameters (force, position, or both) and stiffness regulation and a choice of order. Force feedback is derived from the control force between the limb and the external load. The modulation of the stimuli applied to each muscle is based on a method employed previously; the output of the controller is mapped to the values of the stimuli to be applied to each muscle. With this scheme, the degree of coactivation of the muscles can be controlled in a preprogrammed manner. Mathematically, the simulation is broken down into two parts: the controller calculations and the estimation of the muscle are carried out at discrete times corresponding to the stimulus period; between each stimulus, the muscle and load equations are integrated using a variable step-size Runge-Kutta method. The simulation is very general and almost all parameters can be altered freely and specific questions are being addressed. The first is the performance of a control system under different constant external loads and the second is the performance at load transitions such as take place when reaching for and grasping an object. Supported by NINDB Contract N01-NS-6-1182 and NICHD Grant G080630011B.

470.4 SELECTIVE ACTIVATION OF SMALL MOTOR AXONS BY SPIRAL CUFF ELECTRODE AND QUASI-TRapeZOIDAL STIMULATION PULSES. L.P. Fearn, and J.T. Martinek. Applied Neural Control Lab., Dept. of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106.

We have found a method to activate electrically small fibers without activating large fibers in the same nerve trunk. The method utilizes a previously developed technique for generating unidirectionally-propagating action potentials (van den Honert, C., et al, Science, 261:121, 1979). Quasi-trapezoidal stimulus pulses were delivered by a tripolar cuff electrode to excite all nerve fibers of different diameters under the center-positioned cathode while blocking selectively large fibers under the side-positioned anodes. The quasi-trapezoidal shaped current pulses, with a peak of 350 μA and a exponential decay, insured the biophysical propagating action potentials and prevented the anodal break effect. The tripolar configuration of the electrode restricted current inside the cuff and prevented the virtual cathode effect. The self-curving spiral structure made the cuff electrode easy to install and reduced the likelihood of mechanical stimulation to the nerve (Napier, G.G., et al, Soc. Neurosci. Abstr., 12:1307, 1986).

Acute experiments were performed on 13 adult cats. The branch of the sciatic nerve innervating the medial gastrocnemius was surgically isolated for a length of 3-4 cm to install the spiral cuff electrode. After laminectomy, action potentials were recorded from L7 ventral roots. As the stimulus amplitude increased from zero to about 0.1 mA, the compound action potential amplitude increased rapidly to the maximum with several peak representing alpha and gamma fiber groups of different conduction velocities. When the stimulus current was raised beyond 1 mA, the amplitude of the compound potential decreased to decay with the early peaks in the alpha complex being suppressed first, sparing the slower ones. At even higher current amplitude of about 3 mA, the whole alpha complex faded, leaving only the gamma components. After compound action potential recording, the L7 ventral root was teased until a small fascicle containing only two to three alpha fibers of different conduction velocities was obtained. The recordings showed that the faster components of alpha fibers would always be suppressed while sparing conduction of the slower ones.

This method has been used to activate selectively small motor units in skeletal muscles in an attempt to obtain a close-to-foot switch recruitment order in electrically activated muscles. It may also be used for selective activation of small sensory fibers in chronic periphery nerves. In comparison with DC block, high frequency block, and triangular voltage pulse block with hook electrodes, this method has the merits of not causing nonspecific firing, less charge injection and easy to implement in chronic application. Supported by the Neural Prosthesis Program, NID-NEI contract No. 80-09-6-2344.

Restoration of voluntary hand grasp and elbow extension in the tetraplegic upper extremity has been achieved with tendon transfers. These procedures involve transfer of a voluntary motor into the insertion of a paralyzed muscle. Two patient groups were evaluated. The first had weak C6 function, with only the ECRB, ECRL, brachioradialis (BR), biceps and brachialis intact distal to the shoulder. The BR was transferred to the FD to provide finger flexion, the ECRL (via a graft from the FDG) to the APB to provide thumb opposition, and the posterior head of the deltoid to the triceps. The second group had stronger C6 function, with voluntary control of the PT, FCR, and triceps muscles in addition to those listed above. These subjects had the BR as the antigravity flexion, and the PT for thumb opposition.

Three patients in each group were evaluated at least one year after surgery. Thumb and finger forces and muscle firing patterns were measured with external transducers and electromyography.

Significant differences in thumb pinch and finger grasp strengths exist between the two subject populations. With the elbow 135° extended and the wrist neutral, the average pinch strengths were 10.6 (s.d.+2.9) N for the weak and 26.4 (s.d.+7.4) N for the strong subjects. The average grasp strengths were 13.0 (s.d.+2.1) N and 23.3 (s.d.+10.3) N. One cause of the difference in thumb force is likely related to the use of different motors. The PT has a greater flexion torque fraction (35% PT) than the ECRB (ECR+ECRL). In addition, the ECRL is expected to exert more force (Brand, P.W., Hand Surgery, 6(3):209, 1981). The differences in finger pinch and grasp forces when muscle length was changed by varying the wrist and elbow positions.

New phasic patterns of muscle activity were observed in the transferred muscles. All motor units were active in the hand during the interval between the first and second phase of the tap. The subject was instructed to direct all voluntary control to the forearm. Two paradigms were used: (1) the subject was instructed to flex the elbow after the first phase of activity; and (2) the subject was instructed to flex the elbow after the second phase of activity.

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470.6 REACTION AND MOVEMENT TIMES IN ELDERLY SUBJECTS AND PATIENTS WITH PARKINSON'S DISEASE. J. D. Brown and J. D. Cooke, Dept. of Clinical Neurological Science and Dept. of Physiology, University of Western Ontario, London, Ontario, Canada.

Difficulty in initiating movements and slowness of movement are commonly observed sequelae of Parkinson's disease. In the extraneous tasks may result in the complete absence of movement (akinesia) in these patients. This akinesia may, however, be overcome in some situations by an appropriate sensory cue. This cue was given to the patient for movement initiation. We have studied the effect of different sensory cues on movement initiation in Parkinsonian patients. Since motor ability changes with age, comparative studies were performed on age and sex matched normal subjects.

Subjects performed 40 deg flexion/extension movements about the elbow between two fixed targets. Subjects were instructed to initiate movement as quickly as possible upon cue presentation and to move to the new target "as quickly and as accurately" as possible. Visual, kinesthetic (limb perturbation) and vibratory (vibration applied to the forearm) cues were given. The interval between successive cue presentation varied randomly from 1-3 sec. A total of seven 40 deg flexion/extension movements about the elbow were performed.

Overall, there was little difference in reaction times between the patients and the control subjects. Reaction times did not vary consistently with the type of sensory cue in either group. Total movement time and the time from movement initiation to peak velocity were generally greater in the patients. However, different subjects often employed different strategies for making the movements. Some subjects overtht the target area to make a rapid retury movement into the target. Others slowed well before the target was reached, entering the target with a slow, prolonged corrective movement. The former subjects generally had fewer movement times and times to peak velocity than the latter.

The data suggest that the changes in motor performance seen in the elderly parallel those seen in patients with Parkinson's disease. The data also suggests that movement initiation and performance in Parkinsonian patients are dependent on the nature of the simple cues for movement. Improvement in akinesia which was reported upon voluntary movements in the elderly may be related to the nature of the cues presented rather than to the specific sensory modality of the cue.

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470.7 VOLUNTARY OROFACIAL MOTOR CONTROL IN SCHIZOPHRENIA AND TARDIVE DYSKINESIA. M.J. Caligiuri, M. Harris*, R. De Frese*, J. D. Hammond*, P. B. Nutter* and G. H. Kraft*.

Orofacial dyskinesia is present in over 80% (Jeste and Wyatt, 1982) of patients with tardive or spontaneous dyskinesia. Yet, instrumental approaches have generally not been applicable to the assessment of motor control in schizophrenia or in patients with TD. The present study reports the results of an investigation using strain gauge instrumentation to quantify voluntary control of labial, mandibular and lingual movements in schizophrenic patients with and without TD. Subjects had normal static postural control. A total of 12 patients (treated and untreated) and 12 age and sex matched controls were studied.

Subjects were instructed to maintain maximal orofacial control for at least 2 min. The results of the present study are that patients with schizophrenia are less able to maintain normal control of one orofacial muscle group than normal controls.

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470.8 GAPs IN EMG ACTIVITY DURING ISOMETRIC WRIST FLEXION AND EXTENSION BY CHRONIC CVA PATIENTS. G. S. Pits, C. C. Hammond*, P. B. Nutter* and G. H. Kraft*.

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"Catchy" motor unit recruitment is commonly recognized during clinical assessment of upper motor neuron deficits. The phenomenon has not been applied to the assessment of motor control in schizophrenia or in patients with TD. The present study reports the results of an investigation using strain gauge instrumentation to quantify voluntary control of wrist flexion/extension by chronic CVA patients. The data also suggests that movement initiation and performance in Parkinsonian patients are dependent on the nature of the simple cues for movement. Improvement in akinesia which was reported upon voluntary movements in the elderly may be related to the nature of the cues presented rather than to the specific sensory modality of the cue.

Supported by the Physician's Services Foundation Inc. of Ontario.
470.9  ISOMETRIC TREMOR AND MOST RAPID VOLUNTARY CONTRACTIONS IN PATIENTS WITH HEREDITARY MOTOR AND SENSORY NEUROPATHY (HMSN). E. Logigian*, R. Bahrmer*, R. Rufner* and B.-J. Freund* (Spons: R. Young), Dept. of Neurology, University of Dusseldorf, D-4000, Dusseldorf i, FRG.

In patients with HMSN types I (N=5) and II (N=5), we investigated isometric force tremor of index finger abduction recorded in steps from 0 to 70% maximal voluntary force (MVF), while simultaneously measuring rectified EMG from the first dorsal interosseous muscle (FDI). Peak tremor frequencies were then determined. In addition, we isometrically recorded most rapid voluntary contractions of index finger abduction over a range from minimal to maximal peak force, while simultaneously measuring rectified EMG from FDI. For each patient, the peak force (PF) of each contraction was plotted vs. its contraction time (CT) to obtain the slope (CT/PF) of this linear relation.

Peak tremor frequencies were significantly higher in HMSN II patients than in those with HMSN I matched for MVF of index finger abduction. In those who underwent needle examination, EMG and tremor peak frequency were positively correlated. Similarly, CT/PF was significantly lower in HMSN II than in matched HMSN I patients, but there was no significant difference between the two groups in CT of index finger abduction evoked by supramaximal ulnar nerve stimulation. In both groups, CT/PF and FDI EMG burst length were negatively correlated with MVF of index finger abduction.

Higher tonic and maximal SMU firing rates may underline higher peak tremor frequencies and shorter voluntary contraction times, respectively, in HMSN II compared to matched HMSN I patients.

During most rapid voluntary isometric contraction, weaker patients with HMSN of either type employ longer agonist EMG burst length (and CT), than do stronger patients, to reach a given peak force.


When patients with cerebellar incoordination attempt to perform a relatively slow tracking task they often generate inappropriately high velocity movements. This study was undertaken to determine whether tracking performance could be improved by modifying the mechanical properties of a manichand used for manual tracking of a visual target.

Four patients with unilateral cerebellar lesions and four age-matched control subjects were presented with a visual display of a target moving horizontally across a screen in an unpredictable pattern at velocities of 0-30 degrees/sec. They were required to track the target by flexing and extending the wrist. After a learning period to familiarize the subjects with the apparatus, the viscoisity, stiffness, and inertia of the apparatus were systematically altered. Viscous resistance and stiffness were provided by feeding back the velocity and position, respectively, to the amplifier that powered a torque motor. Inertia was altered by adding weights to the manipulandum. The cumulative difference between the signals representing wrist position and target position provided a measure of error for each 20 second trial. The amount of EMG activity in flexors and extensors was also measured, and the degree of coactivation estimated. Signals representing target and wrist position were differentiated to examine movement velocity.

When patients performed the tracking task with the affected arm, error scores were significantly higher (p<0.005) than in the clinically unaffected arm. Increasing viscosity improved tracking accuracy in the affected arms of patients (p<0.01), but not in the clinically unaffected arm nor in control subjects. Increasing the stiffness produced no consistent change in error scores. Addition of inertia either made no significant change in the accuracy, or increased the error.

These results suggest that application of a viscous interface may dampen unwanted movements and improve accuracy of performance in patients with cerebellar incoordination.

Supported by the Alberta Heritage Foundation for Medical Research and the MRC (Canada).


This study sought to determine if targeted (conventional) electromyographic (EMG) feedback training (N=13) was superior to a novel task of motor (reference) copy (N=13) in producing functional and neuromuscular changes among chronic stroke and head injured patients. Targeted training consisted of usual channel monitoring of specific muscle groups in the hemiplegic limb, while motor copy required controlled activity of bilateral homologous muscle groups for which a visual representation of a muscle signal from the uninvolved muscle served as a template to be matched from output of the involved muscle. Thirty treatments followed specific protocols with functional and neuromuscular evaluations given at 5 baseline sessions prior to treatment, after every 10 treatment sessions and at 3 and 6 month follow ups. Both groups showed significant improvements in time and force measures of most of the 12 functional tasks with the target muscle training group showing more significant changes among tasks overtime (Felman 2 way ANOVA). Between group analyses (Non-Shifts) indicates that among 7 tasks the target trained group showed more significant changes than motor copy.

Both groups demonstrated significant increases in active shoulder flexion range of motion whereas the targeted training group also showed significant increases in active wrist flexion and finger extension. The targeted training group also demonstrated a significant increase in mean EMG activity of anterior deltoid, biceps and wrist extensor musculature. These changes in neuromuscular characteristics may account for the greater number of significant functional improvements on the targeted training group.

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470.12  PARADIGM FOR THE CLINICAL ASSESSMENT OF QUANTITATIVE JOINT FUNCTION. P. Weiss, B.E. Kearney, I.V. Hunter, B. Morier* and B.Dennissen*, School of Physical and Occupational Therapy and Biomedical Engineering Unit, McGill University, Montreal, Canada, H3G 1Y5.

Although it is well recognized that neuromuscular disease can severely disrupt functional ability, objective, valid and reliable measures of abnormal muscle and joint behaviour are almost completely lacking. Previous work in this laboratory has resulted in the development of a suite of experimental and analytical techniques, based on system identification, for the rapid, quantitative measurement of the mechanical and reflex properties of the human ankle. The objective of this presentation is to describe a paradigm for the clinical assessment of normal and abnormal quantitative joint function and to report some of our preliminary results.

The subject's ankle is slowly driven (0.05 rad/s) by an electrohydraulic actuator through a trajectory covering most of its range of motion while small amplitudes (0.05 rad) random perturbation of angular position are imposed. The joint position, torque and surface EMGs from the ankle muscles are recorded; these experimental records are divided into short (2.5 s) records within which only small changes of mean joint angle occur. Each segment is analyzed separately to yield a series of dynamic descriptions of the passive ankle mechanics.

The results from one subject are shown in the figure where passive ankle stiffness is plotted as a function of ankle angle. The small and passively stiff highly compliant joint at mid-range positions that becomes considerably stiffer at maximum dorsiflexion is evident.

(Continued...
471.1 THE ANATOMICAL DISTRIBUTION OF PEPTIDES SHOWING PRODYNORPHIN IMMUNOREACTIVITY IN THE CENTRAL NERVOUS SYSTEM OF THE GOLDEN HAMSTER (Mesocricetus auratus). C. Neal, Jr.* and S. W. Newman University of Michigan, Ann Arbor, MI 48109

The anatomical localization of the prodynorphin opioid precursor system has been studied in the rat in detail (Fallon, J.H., JCB, 249:293-336). This system has several of the active opioid sequences present within the original precursor molecule prior to processing. Polyclonal antibodies to dynorphin B (1-13), dynorphin A(1-8) and C-peptide were used in an immunocytochemical study to generate a prodynorphin map of the Golden hamster in coronal and parasagittal brain sections. The C-peptide antibody is directed at the C-terminus of dynorphin B (1-29) and, therefore, is lacking cross-reactivity with the leu-enkephalin amino acid sequence found in the dynorphin opioid peptides. Areas of heaviest prodynorphin cell and fiber staining include the anterior continuation of the hippocampus, lateral septum, dentate gyrus, Ammon's horn (CA1-4), bed nucleus of the stria terminalis (BNST), medial nucleus of the amygdala (MeA), central nucleus of the amygdala, medial preoptic area (MPOA), suprageniculate nucleus, retrochiasmatic suprageniculate nucleus, ventromedial nucleus of the hypothalamus, paraventricular nucleus of the hypothalamus, lateral hypothalamus, reticular formation, peripeduncular nucleus, nucleus ambiguus, accessory motor nucleus of the fifth nerve, and the solitary nucleus. Heavy fiber and terminal staining is observed in many of the above areas as well as in the central pallium dentate gyrus, arcuate nucleus of the hypothalamus, paraventricular nucleus of the thalamus, parabrachial nucleus, substantia nigra (SNR), medial habenula and nucleus of the solitary tract.

Though the locations of these prodynorphin-containing neurons are very similar in general to staining generated in the rat, several species differences do appear to exist. In the globus pallidus, caudate-putamen and SNR, known areas of prodynorphin cell and/or fiber staining in the rat, weak staining is observed in the hamster. In contrast, in the MPOA, BNST, and MeA, areas that lightly stain for prodynorphin-containing fibers and cells in the rat, heavy cell and fiber staining is observed in the hamster. The staining variations may reflect important differences in the functional significance of MPOA, BNST, and MeA between the two species. In the hamster, but not the rat, chemoafferent pathways through the MeA & BNST to the MPOA are essential for normal male sexual behavior.

(supported by NIH MH020269 to SNR)
**471.3** DOPHRIN A-IMMUNOREACTIVE NEURONS IN CA FIELDS OF RAT HIPPOCAMPUS ARE REVEALED BY 6-HYDROXYPHEPHEDRINE. J.P. Holcomb and J.R. Lovinger, Dept. of Anatomy, North Carolina University School of Medicine, Greensboro, NC 27410.

Dophrin immunoreactivity has been described by several groups to be located in the hippocampal formation exclusively in the dentate granule cell-mossy fiber system. In contrast, electrophysiologists have recorded substantial effects of endogenously acting dophrin in the CA1 field in vivo and in vitro. In normal rats perfused with a 4% paraformaldehyde solution and with or without 1% glutaraldehyde, the dophrin immunoreactive labelling is not seen. One week after intracerebroventricular (200 μg/10 μl) or intracerebroventricular (5 μg/4 μl) injections of 6-hydroxyphephedrine (6-OHDA), numerous dophrin A-immunoreactive cell bodies were visualized in CAl-4, few more in CA5, but never in CA2-3. The cell bodies are located principally in stratum radiatum (s.r.) and stratum oriens (s.o.); however, some somas are oriented close to or in the pyramidal cell layer. Neurons in s.r. are large and have multiple, long, radial processes extending in an apical direction through s. lacunosum to the edge of (but not penetrating) stratum moleculare and in a basal direction to the pyramidal cell layer. Some somas are in the alveus; they have a multipolar or horizontal configuration with many processes extending in parallel and/or perpendicular to the pyramidal cell layer. Fewer neurons were seen in CA3 and CA4 and none were seen in the dentate gyrus except for occasional immunopositive granule cells which have been described previously. These data raise the possibility that dophrin metabolism in cell bodies in hippocampal formation are normally inhibited by norepinephrine. Electrophysiological recordings of CA1 pyramidal cell activity in the presence of different combinations of opioid and norepinephrine agonists and antagonists may yield some insights into the interactions between these two neurotransmitter substances in the hippocampal formation. -Supported by DA 03892.

**471.4** OPIOCORTIN AND CATECHOLAMINERGIC PROJECTIONS TO PERIAQUEDUCTAL GREY AND RAPHE NUCLEUS. L. Simt, E. Lund, and S. Joseph.

Neuroendocrine Unit, Univ. of Rochester, Rochester, NY 14642.

Anatomical and physiological evidence has implicated the periaqueductal grey (PAG) and certain raphe nuclei in opioid analgesia. Electrophysiological studies have shown that microinjection into ventral and lateral PAG, a rich region in opiate receptors and opioicotphin fibers, elicits a profound, naloxone-reversible analgesia. Recent studies imply that these two nuclei (PAG and RAP) are both involved in the analgesia. The present study was undertaken to evaluate whether opioicotphin projections to PAG and raphe nuclei emanate from arcuate or NTS raphe opiocortin neurons. Whole ganglia-conjugated horseradish peroxidase (WGA-HRP) was selectively placed throughout the rostral-caudal extent of the PAG and neighboring raphe regions as well as medullary raphe nuclei. Forty-eight hours later, rats were either perfused with Kunos's fixative or injected intravenously with 7.7 μl of 4% glutaraldehyde (WGA-HRP) was selectively placed throughout the rostral-caudal extent of the PAG and neighboring raphe regions as well as medullary raphe nuclei. Forty-eight hours later, rats were either perfused with Kunos's fixative or injected intravenously with 7.7 μl of 4% glutaraldehyde. WGA-HRP activity was demonstrated using routine immunocytochemistry intensified with nickel DAB rendering a black precipitate. The immunocytochemistry intensified with nickel DAB rendering a black precipitate. The immunocytochemistry intensified with nickel DAB rendering a black precipitate. The immunocytochemistry intensified with nickel DAB rendering a black precipitate. These precipitates were stained with antisera to met-enkephalin and dynorphin A. The specificity of these precipitates was determined by immunoelectrophoretic studies. S.M. Prouty and I.S. Zagon.  Dept, of Anatomy, The M.S. Hershey Med. Ctr. of The Pennsylvania State University, Hershey, PA 17033.

Endogenous opioid systems (endogenous opioids and opioid receptors) are known to regulate important aspects of neurodevelopment. In a previous light microscopic study (Science 227:1049-1051, 1985), met-enkephalin immunoreactivity was localized in specific cell populations of the developing rat cerebellum, but not in their adult neuronal counterparts (e.g., internal granule neurons=IGL). This raises the possibility that met-enkephalin metabolism in cell bodies in the cerebellum are normally inhibited by norepinephrine. Electrophysiological recordings of developing rat cerebellum in the presence of different combinations of opioid and norepinephrine agonists and antagonists may yield some insights into the interactions between these two neurotransmitter substances in the cerebellar development. In the present study, we addressed the question of the ultrastructural localization of endogenous opioids and developing adult rat cerebellum using immunocytochemistry.

Sprague-Dawley rats, 11 and 40 days of age, were anesthetized and perfused intracardially with 4% paraformaldehyde, 0.25% glutaraldehyde in 0.07M Sorenson's Phosphate buffer with sucrose (pH 7.4, 25°C) for 5 min. Brains were removed, postfixed in primary fixative for 3 hr, followed by postfixation in the primary fixative without glutaraldehyde for 12 hr. Vibratome sections (120 μm) of the cerebellum, in both sagittal and coronal planes, were stained with antisera to met-enkephalin (ImmunoNuclear), followed by goat-anti-rabbit IgG conjugated to alkaline phosphatase, and processed in diaminobenzidine. Sections were embedded in Epon. Preliminary observations indicated the presence of met-enkephalin immunoreactivity in EGL cells of 11-day old animals. This activity was localized in the cytoplasm, and was not in the nucleus. It was associated with mitochondria, the plasma membrane, and aggregates in the cytoplasm. Immunoreactivity was not detected in parallel fibers of the molecular layer. Some immunoreactive product was found in the soma of IGL cells. Purkinje cells were filled with met-enkephalin and displayed prominent staining in soma and dendrites, and was associated with organelles (except the nucleus) and dendritic spines. Control sections showed no immunoreactivity. Observations of 40-day old rats revealed little staining in either Purkinje or IGL neurons. These results agree with and earlier reports that endogenous opioids such as met-enkephalin play a specific role in cerebellar development, and show that opioid activity is intracellular and associated with discrete cytoplasmic entities. Moreover, these observations are consistent with the hypothesis that endogenous opioids are neurotrophic factors involved in cell proliferation and differentiation. Supported by NIH grants NS-20500 and NS-20623.

**471.5** NET-ENKEPHALIN IMMUNOREACTIVITY IN DEVELOPING RAT CEREBELLMUM: PRELIMINARY IMMUNOELECTRON MICROSCOPIC STUDIES. S.H. Probst and E.S. Haggard, Dept. of Anatomy, The M.S. Hershey Med. Ctr. of The Pennsylvania State University, Hershey, PA 17033.

Net-enkephalin immunoreactivity in the developing rat cerebellum, but not in their adult neuronal counterparts (e.g., internal granule neurons=IGL). This raises the possibility that net-enkephalin metabolism in cell bodies in the cerebellum are normally inhibited by norepinephrine. Electrophysiological recordings of developing rat cerebellum in the presence of different combinations of opioid and norepinephrine agonists and antagonists may yield some insights into the interactions between these two neurotransmitter substances in the cerebellar development. In the present study, we addressed the question of the ultrastructural localization of endogenous opioids and developing adult rat cerebellum using immunocytochemistry.

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**471.6** CHARACTERIZATION OF OPIATE AND SIGMA RECEPTOR TYPES ON THE NCB-20 CELL MEMBRANE. R. Zahniser and R.S. Zukin. Dept, of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

The neurokinin X Chinese hamster hybrid Clone cell line NCB-20 is the only cell line known to contain benzomorphon-specific sites in addition to delta opioid receptors in the developing rat cerebellum, but not in their adult neuronal counterparts (e.g., internal granule neurons=IGL). This raises the possibility that net-enkephalin metabolism in cell bodies in the cerebellum are normally inhibited by norepinephrine. Electrophysiological recordings of developing rat cerebellum in the presence of different combinations of opioid and norepinephrine agonists and antagonists may yield some insights into the interactions between these two neurotransmitter substances in the cerebellar development. Supported by DA 03892.
**471.7 LABELING OF THE HALOPERIDOL-SENSITIVE SIGMA RECEPTOR IN NC203**

**SYNERGY NEUROBLASTOMA CELLS WITH [3H]-(1,3)-DI-ORTHO-TOLYL-GUANIDINE AND IDENTIFICATION OF THE BINDING SUBUNIT BY PHOTAFFINITY LABELING WITH [3H]-3-{2-AZIDO-1-(3-BENZYL)-INDOXYL-5-METHYLRESORCINOL}

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**[3H]-(1,3)-Di-ortho-tolyl-guanidine** selectively labels haloperidol-sensitive sigma sites in guinea pig brain membranes (Weber et al., PNAS 81: pp.1-6, 1984). Binding studies with [3H]DTG in NC203 cell membranes confirmed that the sigma site has high affinity for this ligand (Kd = 27+2 nM) and moderate binding values of 70±5 pmol/mg protein, respectively. Labeling with [3H]DTG completely displaced binding of 0.9 nM [3H]3-PPP (a selective radioligand for sigma receptors previously analyzed in this cell line, Largent et al., J. Pharmacol. Exp. Ther. 269, pp.183-187, 1994). Unlabeled (+)-3-PPP, in turn, displaced binding completely. In direct competition experiments, the drug potency profiles for (+)-3-PPP and (+)-DTG binding in NC203 cell membranes were different, despite the identical drug profiles in guinea pig brain membranes (Weber et al., PNAS 83, pp.1-6, 1984). In general, the benzomorphan drugs and the BuChophenol, haloperidol, were less than one tenth as potent as inhibitors of [3H]DTG binding as compared to (+)-3-PPP binding. In NC203 cell membranes, [3H]DTG binding sites were determined. Whole sea anemone and planaria, the ganglia and ventral nerve cords of earthworm and crayfish, and either regions of neural tissue from representatives of thirteen animal classes and phyla were analyzed. The present results indicate that both the a and PCP binding sites for (+)-3-PPP and DTG. (+)-3-PPP and DTG were competitive inhibitors, increasing the Kd while having no effect on B. (+)SKF 10,047 interacts via a different site. This observation is consistent with the differential photopharmacological characterization of (+)-3-PPP and (+)-DTG binding to human cerebellar membranes indicates that their sites of interaction with the receptor pharmacologically differ.

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**471.8 THE SELECTIVE SIGMA LIGAND [3H]-DTG BINDS TO SITES IN HUMAN AND GUINEA PIG CEREBELLUM WITH DISTINCT PHARMACOLOGICAL PROFILES.**

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We have undertaken a comparison of the affinities of (+)-3-PPP and DTG to [3H]-TCP binding sites in guinea pig whole brain and cerebellum and human cerebellum. We chose to study cerebellar cytoarchitecture makes it amenable to electrophysiological investigation and rodent cerebella contain a large preponderance of sigma compared to PCP binding. We performed a phylogenetic study of the distribution of these sites in various species, comparing to [+]-TCP binding site for (+)-3-PPP [Sonders et al. (1983) TINS, in press].

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**471.9 PHENOCYCLINE DISTRIBUTION OF HALOPERIDOL-SENSITIVE SIGMA AND PHENOCYCLINE BINDING SITES IN THE NERVOUS SYSTEM.**


Benzomorphan and certain arylcycloalkylamines may mediate psychotomimetic effects through their interaction with specific receptors in the brain. These sites include sigma (σ) and PCP (Phencyclidine) ligand binding sites, which can be labelled with [3H]HALOPERIDOL and [3H]PHENCYCLIDINE, respectively. Although σ and PCP sites differ in pharmacology and anatomical distribution, the possibility of a functional interaction between these two sites is open to gain insight into the origin and possible relation between σ and PCP sites, as well as the nature of the distribution of the affinities and affinities of the specific binding homogenates of neural tissue from representatives of thirteen animal classes was determined. Where no anxious and planaria, the ganglia and ventral nerve cords of earthworm and crayfish, and either regions or whole brains of the species were examined. The present results indicate that the a and PCP binding sites are phylogenetically old and are distributed throughout the animal kingdom. Furthermore, the affinities of specific ligands for each of the binding sites appeared similar across several species of vertebrates.
471.1 THE SIGMA-SELECTIVE PHOTOOAFFINITY LIGAND [3H]AZIDOTO- DICTYOLYGUANIDINE SPECIFICALLY LABELS A 29 KD POLYPEPTIDE FROM GUINEA PIG BRAIN MEMBRANES M.E. Kavanagh*, B.A.C. Trotter†, J.W. Keana*, and E. Weber†. Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201 and †Department of Chemistry, University of Oregon, Eugene, OR 97403.

The haloperidol-sensitive sigma receptor is a candidate for mediating the psychotomimetic effects of benzomorphans in humans. In addition to the stereospecificity of the benzomorphans, the receptor binds [3H]scopolamine, or GABA at the same concentration. These results completely blocked by the sigma ligands DTG, (+)-3-PPP, (+)-pentazocine, and haloperidol at a concentration of 10-20 nM. Morphine and DADL-enkephalin at concentrations under 10 nM lowered [3H]aza-endorphin binding (0.2 nm) by about 25-30%. However, the inhibition of the two compounds was not additive. In the presence of one ligand, the other failed to lower binding. The remainder of the binding was more sensitive to a-endorphin than either of the other two ligands. [3H]aza-endorphin binding at all, implying that at this concentration of radioactive material no delta receptors were labeled. In addition, the existing affinity. There is no evidence of delta labeling and mu, labeling also seems unlikely. Having characterized the binding of the ligated ligand, we used this low concentration of [3H]aza-endorphin in crosslinking studies and ran SDS-PAGE gels. Our results are similar to those in other groups, with the major band being at about 60-65,000 daltons and several additional bands corresponding to lower molecular weight species. However, our binding studies raise major questions regarding the published identification of these bands with specific classes of opioid receptors.


Sera from two patients with major depressive disorder and antibodies to 8-endorphin (Roy, B.F., USA 83:8739, 1986) are shown to have binding antibodies which specifically bind to mammalian brain membranes. The antibodies to 8-endorphin inhibited 8-endorphin binding to rat brain membranes and were demonstrated to be directed against receptors to 8-endorphin. Identical recognition of 8-endorphin binding to rat brain membranes and was demonstrated. Antibody was eluted from sheep anti-mouse immunoglobulin G column and incubated with a crude P2 membrane fraction from rat brain in a buffer containing 50 mM potassium phosphate, pH 7.4, and protease inhibitors for 60 min at 25°C. Then 1 nM [3H]8-endorphin was added and incubation was carried out for an additional 60 min at 25°C. Incubations were terminated by filtration through glass fiber filters. Nonspecific binding was determined using 10 μM levarterenol. Autoantibody binding to 8-endorphin was reduced by binding of [3H]8-endorphin to the P2 membrane fraction of rat brain 89%, while homologous anti-idiotypic antibody reduced binding by 70%. Neutral Ig which was similarly treated had negligible competitive effects at equivalent concentrations. The idiotypic specifically inhibited the ability of anti-idiotypic to block [3H]8-endorphin binding to rat brain opiate receptor sites. These antibodies indicate that the anti-idiotypic antibody binds to opioid receptors. The ability of anti-idiotypic antibody to recognize membrane proteins was assessed by Western Immunoblot analysis. Solubilized rat brain membrane proteins were resolved by two-dimensional polyacrylamide gel electrophoresis and blotted onto nitrocellulose. The blots were probed with autoantibody to 8-endorphin followed by incubation with peroxidase-coupled goat anti-human IgG. A neutral protein of 60 kDa was detected. This molecular weight is consistent with the protein corresponding to that of the major protein labelled by iodinated 8-endorphin in cross-linking studies (Howard, A.U., J. Biol. Chem., 260:10833, 1985). Additional bands of 116 and 110 kDa were detected in one-dimensional gels when [3H]8-endorphin antibody was used as the method of visualization.

The anti-idiotypic is potentially directed against receptors on f and b cells promoting the production of anti-8-endorphin antibody may mediate interactions between parallel regulatory networks within the immune system and the CNS. Idiotypic idiotypic networks for brain peptides may contribute to psychiatric disorders.


Previously, our group has studied [3H]aza-endorphin binding in brain extensively. In these studies, we found that [3H]8-endorphin labeled two classes of sites with high affinity (K<1 nM). One corresponded to the mu site, while the other was selective for 8-endorphin and did not appear to correspond to either morphine-selective mu or delta sites. In view of the extensive use of [3H]8-endorphin to identify opioid binding sites through crosslinking approaches, we have examined its binding to brain membranes and examined its crosslinking. As with the tritiated material, [3H]8-endorphin bound extensively to glass fiber filters and we therefore used a microfuge assay, although treating the filters with polyethyleneimine was useful. Saturation studies demonstrated a high affinity component with a Kd value of approximately 0.7 nM, very similar to that reported previously with the tritiated material. Raising the concentration of 8-endorphin further revealed additional binding sites with lower affinity (10-20 nM). Morphine and DADL-enkephalin at concentrations under 10 nM lowered [3H]8-endorphin binding (0.2 nm) by about 25-30%.

However, the inhibition of the two compounds was not additive. In the presence of one ligand, the other failed to lower binding. The remainder of the binding was more sensitive to 8-endorphin than either of the other two ligands. DPDPE at concentrations up to 20 nM, over 25-fold higher than its Kd for delta receptors, failed to lower [3H]8-endorphin binding at all, implying that at this concentration of radioactive material no delta receptors were labeled. In addition, the existing affinity. There is no evidence of delta labeling and mu, labeling also seems unlikely. Having characterized the binding of the ligated ligand, we used this low concentration of [3H]8-endorphin in crosslinking studies and ran SDS-PAGE gels. Our results are similar to those in other groups, with the major band being at about 60-65,000 daltons and several additional bands corresponding to lower molecular weight species. However, our binding studies raise major questions regarding the published identification of these bands with specific classes of opioid receptors.


Anti-idiotypic, anti-receptor antibodies raised against antibodies to receptor ligands, provide a means of receptor isolation and characterization. These antibodies are currently undergoing studies. A monoclonal anti-idiotypic antibody (Ab2) from monoclonal anti-morphine antibodies (mAbl) using conventional hybridoma methods. In one study, the Ab2 was shown to display stereospecificity and a greater affinity for opioid alkaloid agonists than antagonists. None of the enkaphalins tested however, reacted with mAbl. Hybridoma culture supernatants were screened with a newly developed solid phase radioimmunoassay (RIA), based on the displacement of radiolabelled morphine from mAbl with Ab2. One of the Ab2s that gave a positive RIA also competed with opioid ligands for rat brain opioid receptors. SSB-PAGE revealed the Ab2 to be highly purified after successive affinity and Protein A-Sepharose chromatography. In dose-dependency displacement experiments, molecules either opioid alkaloids or peptides as radio-ligand, this preparation displayed picomolar affinity for rat brain opioid receptors. Less than 15 μg mAbl completely inhibited specific opioid radioligand binding to both soluble and membrane-bound opioid receptors. These results indicate that we have generated an anti-idiotypic antibody that specifically interacts with rat forebrain opioid receptors. Supported by a Fogarty Senior International Postdoctoral Fellowship (to CJC). NIH grant MH 40005 and NSF grant 85-08934.
SATURDAY AM OPiates, ENDORPHINS and ENKEPHALINS: ANATOMY and CHEMISTRY

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THE EFFECTS OF pH ON THE POTENCY OF SELECTIVE INHIBITORS OF MEMBRANE-BOUND ENKEPHALINASE (encephalins, a(11) from rat kidney cortex) and thiorphan, differ substantially. This prompted us to examine the functional role of endogenous enkephalins in the regulation of the dopaminergic neurons since opiates interact with enkephalin receptors which likely play an inhibitory action on dopamine transmission. We thus further investigate the behavioral effects and the common properties of dopamine agents such as amphetamine, haloperi-dol, apomorphine and opioid agents such as morphine, DLET, DAGO and ketorphan on the intracranial self-stimulation behavior (ICSS) obtained through chronic electrodes implanted into the medial forebrain bundle (MFB) after lateral ventricle (ICV) or nucleus accumbens injections. The results indicate that ketorphan (70 nM) and d-amphetamine (1 nM), directly ICV, induced an increase in ICSS lasting for 45 min. This facilitatory effect of MFB stimulation after ketorphan injection was completely antagonized by 70 nM naloxone when co-administered. The ICV injection of morphine (2.5 nM) and an opioid agonist DAGO (300 nM) led to a drastic ICSS decrease whereas 300 nM DLET (a delta agonist) significantly enhanced the ICSS rate (the IC50 value for a delta agonist) DAGO elicited a similar behavioral profile when administered into the nucleus accumbens, i.e., a decrease in ICSS. DLET (1,000 nM), on the contrary, led to a slight non significant ICSS increase. Ketorphan as well as d-amphetamine or apomorphine, induced a significant decrease in ICSS after accumbens injections. The microinjection of the neuroleptic haloperidol ICV or intra-accumbens strongly blocked the rate for ICSS additionally supporting the direct linkage of the to total enkephalinase activity, however kidney was devoid of this amine protease. The soluble and membrane bound forms of the purine-sensitive aminopeptidase, though were found to have the same molecular weight as determined by SDS-PAGE and size exclusion chromatography. The anti soluble aminopeptidase antisera reacted with both enzyme forms on immunoblot and inhibited both enzyme forms with identical inhibition curves.

COMPARISON OF SOLUBLE AND MEMBRANE-BOUND FORMS OF ENKYPHALINASE: ACTIVITIES AND THE EFFECTS OF D-OPIATE ANTAGONISTS ON THE DISTRIBUTION OF ENKYPHALINASE ACTIVITIES

A.M. Desnuelle, Universite de Louvain, 1348 Belgium; B. Roques, Universite Descartes, Paris.

The mesocortico-limbic dopaminergic system, originating from the ventral tegmental area (VTA) and projecting to a wide range of limbic structures including the frontal cortex, olfactory tubercles, lateral septum and nucleus accumbens, have important functions in regulating central reward, exploratory, locomotion behavior and is even thought to be directly implicated in neuro-psychiatric disorders such as schizophrenia, nucleus accumbens and VTA have been shown to display high concentrations of enkephalin and specific opiate receptors (delta and mu) have been identified in close association with both these structures. Ketorphan, a potent inhibitor of enkephalin-degrading enzymes should help to elucidate the functional role of endogenous enkephalins in the regulation of the dopaminergic neurons since opiates interact with enkephalin receptors which likely play an inhibitory action on dopamine transmission. We thus further investigate the behavioral effects and the common properties of dopamine agents such as amphetamine, haloperidol, apomorphine and opioid agents such as morphine, DLET, DAGO and ketorphan on the intracranial self-stimulation behavior (ICSS) obtained through chronic electrodes implanted into the medial forebrain bundle (MFB) after lateral ventricle (ICV) or nucleus accumbens injections. The results indicate that ketorphan (70 nM) and d-amphetamine (1 nM), directly ICV, induced an increase in ICSS lasting for 45 min. This facilitatory effect of MFB stimulation after ketorphan injection was completely antagonized by 70 nM naloxone when co-administered. The ICV injection of morphine (2.5 nM) and an opioid agonist DAGO (300 nM) led to a drastic ICSS decrease whereas 300 nM DLET (a delta agonist) significantly enhanced the ICSS rate (the IC50 value for a delta agonist) DAGO elicited a similar behavioral profile when administered into the nucleus accumbens, i.e., a decrease in ICSS. DLET (1,000 nM), on the contrary, led to a slight non significant ICSS increase. Ketorphan as well as d-amphetamine or apomorphine, induced a significant decrease in ICSS after accumbens injections. The microinjection of the neuroleptic haloperidol ICV or intra-accumbens strongly blocked the rate for ICSS additionally supporting the direct linkage of the to total enkephalinase activity, however kidney was devoid of this amine protease. The soluble and membrane bound forms of the purine-sensitive aminopeptidase, though were found to have the same molecular weight as determined by SDS-PAGE and size exclusion chromatography. The anti soluble aminopeptidase antisera reacted with both enzyme forms on immunoblot and inhibited both enzyme forms with identical inhibition curves.

Studies on the distribution of soluble aminopeptidase activity in seven different rat tissues, using Ala-L-alanine as substrate, showed that the purine-sensitive aminopeptidase is the predominant enzyme activity in brain, heart and skeletal muscle. In spleen, liver and lung this enzyme accounted for approximately 50% of the total aminopeptidase activity, however kidney was devoid of this aminopeptidase.

These results establish a direct relationship between the soluble and membrane bound forms of the purine-sensitive aminopeptidase and the aminopeptidase, like other "enkephalinases" is not brain specific.
DISTRIBUTION OF PROTO-ONCOGENE EXPRESSION IN RAT BRAIN. J. H. Rosen* and J. Villa-Fernandez.

Department of Neurobiology and Neurology, Boston University School of Medicine, Boston, MA 02115.

The presence of proto-oncogenes, such as c-fos, c-myc, and Ha-ras, in rat brain has been reported. However, the distribution and expression of these genes in rat brain, especially during development, has not been extensively studied.

In this study, we used in situ hybridization and immunohistochemistry to examine the expression of c-fos and c-myc in rat brain. The results show that both c-fos and c-myc are expressed in various brain regions, with highest expression in the cerebellum and hypothalamus. These findings suggest that proto-oncogenes play a role in the development and function of the brain.

In situ hybridization was performed using digoxigenin-labeled cRNA probes for c-fos and c-myc. The probes were hybridized to formaldehyde-fixed brain sections, and the hybridization signal was detected using anti-digoxigenin antibodies and the chromogenic substrate 5-bromo-4-chloro-3-indoxyl phosphate. Immunohistochemistry was performed using antibodies against c-Fos and c-Myc.

The results indicate that c-fos and c-myc are expressed in various brain regions, with highest expression in the cerebellum and hypothalamus. These findings suggest that proto-oncogenes play a role in the development and function of the brain.

In conclusion, our results provide new insights into the expression of proto-oncogenes in rat brain and highlight the potential role of these genes in brain development and function.
NEUROFILAMENT MESSERNG RNA LEVELS DECREASE FOLLOWING SCALIC NERVE TRANSECTION. M.K. Goldstein, S. Weiss, R. Lazzarini, P. Schmechel, I. Less and E.M. Schlenker. Lab. of Neuropathology, Univ. of Pennsylvania Med. Sch., Philadelphia, PA 19104. Total RNA was isolated from the left (experimental) and right (control) dorsal root ganglia of Sprague-Dawley rats 3, 7, 14, 21, and 28 days after transection of the left sciatic nerve. Levels of RNA coding for the neurofilament triplet proteins (NF-H, NF-M, and NF-L) were analyzed by Northern and dot blots using 32P-labeled cDNA probes specific for each of the proteins. Autoradiograms reveal a decrease in the RNA level for all three subunits as early as 3 days following transection and continuing for at least 28 days. The results indicate that neurofilament protein synthesis following nerve injury is regulated at the level of transcription.

REGULATION OF RNAs ENCODING THE 28 kD CALCIUM BINDING PROTEIN IN RAT KIDNEY AND CEREBELLUM. T.L. Wood*1 . A.J. Tobin1,2,3, S. Christakos* (SPON: A. Beyer-Mears). Department of Biology, Molecular Biology Institute, and Brain Research Laboratory, Dept. of Neurology, University of Düsseldorf, F.R.G., and Inst. of Genetics, University of Köln, F.R.G., Inst. of Radiation Biology, University of Bonn, F.R.G. Following injury of peripheral nerves the expression of a small group of proteins is sequentially induced or inhibited in the distal nerve stump during Wallerian degeneration and nerve regeneration suggesting the induction/repression of specific genes in nerve repair. In an attempt to identify genes regulated in the regeneration program of rat peripheral nerve we have prepared poly(A)+RNA from mature normal and regenerating (1 week post crush) rat sciatic nerve, respectively. Following the procedure of Okayama & Berg a cDNA library was constructed from poly(A)+RNA of regenerating nerves and 2000 clones were picked for our investigation in microtiter plates. These colonies were screened by differential filter hybridization using 32P-labeled single strand cDNA from regenerating and normal rat sciatic nerve poly(A)+RNA as probes. Further investigation by Northern blotting and sequence analysis of differentially expressed cDNA clones with strong hybridization signals revealed that e.g. Pa (major glycoprotein of peripheral nerve myelin) is repressed at one week post crush while actin, vitaminin, and EF-1 (elongation factor) are highly induced in cells of the nerve sheath. The identified cDNA clones were used to estimate the steady state levels of mRNAs homologous to cDNA sequences which had yielded less intense hybridization signals and could not be identified by VAX/VMS computer analysis. The pattern of differential gene expression observed after nerve injury will be presented.

Supported in part by the Deutsche Forschungsgemeinschaft (SFBZoo, TP CS) and the Ministerium für Wissenschaft und Forschung (MWK) to H.M.W., H.S. is recipient of a Fellowship from the Boehringer Ingelheim Fonds.

MOLECULAR GENETIC ANALYSIS OF PEP-19 EXPRESSION IN DEVELOPING MAMMALIAN BRAIN. L. Sangameswaran* and J. I. Morgan* (SPON: S. Korn). Dept. of Neurosciences, Roche Institute of Molecular Biology, Nutley, NJ 07110. PEP-19 is a 7.6 kDa polypeptide that was discovered in our laboratory as a developmentally regulated neuropeptide from rat brain. Although it has a unique amino acid sequence, it shares a homology with the calcium binding domains of several calcium binding proteins such as S-100, parvalbumin, calmodulin, calbindin and troponin. Thus, PEP-19 may be considered as a small poly-peptide with a single calcium binding domain. By NPL analysis, it was shown that PEP-19 is enriched in the cerebellum and olfactory bulb with smaller amounts present in other regions of the brain. It is localized in Purkinje cells and stellate neurons of the cerebellum as observed by immunocytochemistry with a site-directed polyclonal antibody. We have recently cloned PEP-19 from a sp111 library constructed with poly (A) mRNA from adult rat brain. 18 positive clones were picked after screening 150,000 plaques with the antibody. One of them, Z0D-2, with the longest insert (506bp), was subcloned into phage Z22 and sequenced by the method of Maxam and Gilbert. It contains the entire coding region. The amino acid sequence deduced from the cDNA sequence fitted perfectly with that from the polypeptide. The coding region which is the only open reading frame is followed by 270 nucleotides in the untranslated region.

This clone was used as a probe to determine if PEP-19 mRNA, like the polypeptide, is under developmental regulation in rat brain. The mRNA is detectable as early as embryonic day 17 and increases with age reaching a maximum at 18 days postpartum. We also analyzed mRNA from different regions of the brain as well as several non-neuronal tissues. There is a single species of message in cortex, basal brain, spinal cord, brain stem, olfactory bulb and retina. However, it is undetectable in a variety of nonneuronal tissues tested confirming that this polypeptide is exclusively neural in distribution. The cDNA probe was also used to investigate the mRNA level in cerebellar mutant-mice in an attempt to employ it as a marker to study cerebellar development. The mutants used in this investigation included weaver, lurcher, staggerer, pcd, weaver and lurcher. These data will be discussed in relation to cerebellar development.

We are using in situ hybridization to 5'-labeled antisense RNAs to determine the cellular distribution of the RNAs encoding glutamic acid decarboxylase (GAD) and the 28 kd calcium binding protein (CaBP or Calbindin D) in the brains of mice, rats, and gerrils. These studies address two hypotheses: (1) that altered cellular environments lead to changes in the expression of these two genes; and (2) that increased seizure susceptibility in seizure sensitive genotypes results from the altered expression of two genes in the hippocampus and substantia nigra.

In normal mice, GAD mRNA is found in especially high concentrations in cells in the following brain regions: cerebellum, hippocampus, reticular nucleus of the thalamus, cortex, inferior and superior colliculus, substantia nigra pars reticulata, globus pallidus, red nucleus, diagonal band, olfactory bulb, and zona incerta. In normal mice and rats, CaBP mRNA is found in especially high concentrations in cells in the following regions: cerebellum, hippocampus, dentate gyrus, inferior olives, reticular formation, olfactory bulb, entorhinal cortex, caudate nucleus, amygdala, and vestibular nuclei.

GAD and CaBP RNAs are expressed in distinct but overlapping neuronal types. In the cerebellum, for example, Purkinje neurons contain both GAD and CaBP mRNAs. Basket, stellate and Golgi II neurons, however, contain GAD mRNA but not CaBP mRNA. In the hippocampus, CaBP mRNA is present in a small population of interneurons in all layers, while CaBP mRNA is present at high concentration in the pyramidal cells of CA 1 and CA 2. The granule cells of the dentate gyrus contain high levels of CaBP mRNA, but no detectable GAD mRNA.

We are currently investigating the developmental regulation of these RNAs in normal rodents and in ataxic and seizure susceptible mutants. This work was supported by a grant to AJT from NIMH (MH 47210) and a program project grant to Dr. A.V. Delgado-Escueta (MH 22226) and a program project support to Dr. A.V. Delgado-Escueta (MH 22080). Supported by a USPHS Training Grant in Cell and Molecular Biology (GM 07185).


We have used in situ hybridization to study gene control of cell morphology and function during early development. Our efforts were concentrated on gene expression in the mouse cerebellum where the bulk of the development takes place postnatally between days 8 and 14.

A cDNA library in Agt10 was constructed with total polyA RNA, extracted at day 15 after birth from wild type cerebellum. It was enriched for Purkinje cells by screening techniques with wild type and lurcher RNA preparations (see abstract at this meeting by J. Oberdieck, et al. L7) is a marker for the terminal differentiation of Purkinje cells.

In situ hybridizations were performed on cerebellum sections from wild type mice at different ages after birth: day 1, 4, 10, 14, and 21. The probe, in the experiments reported here, was a nick-translated, tritiated cDNA from a clone chosen for its decreased expression in the lurcher compared to the wild type (L7). Silver grains generated by radioactive decay on a NTB-2 emulsion were counted, over different regions of the brain.

The hybridization is localized in Purkinje cells and their dendrites. There is no labelling found in new born and the counts are maximal at day 8 and 10. Although still present at day 21, they are reduced in the cell bodies. When the silver grains were counted per Purkinje cell and per dendritic tree, the counts per cell are at maximum levels: 79 counts versus 68 at 10 days and 35 at 21 days. In the dendritic tree, the counts are roughly constant: 48 - 40, respectively. All numbers represent average counts of 10 cells.

At age 8 or 10 days, a number of Purkinje cells can be found in the granular cell layer. The amount of silver grains reach their final destination and they have not started sprouting their dendrites. The lurcher label. We have other clones which hybridize preferentially with Purkinje cells, although not as specifically as L7. Preliminary results indicate that they do not hybridize with the dendritic processes.

L7 represents a DNA sequence involved specifically in the differentiation of Purkinje cells in the cerebellum.

Experiments are in progress to identify at an E.M. level the localization of the signal in the dendrites and several other clones are also being studied.
EXPRESSION OF MYELOID PROTEOLYSIS PROTEINS (PLP AND DM-20) IN THE DEVELOPING HUMAN SPINAL CORD. E.K. Kroonquist*, B.F. Crandall*, K.R. Macklin, and A.T. Campanogoli, Mental Retardation Research Center, U.C.L.A. School of Medicine, Los Angeles, CA 90024.

We have cloned and sequenced a 2956 bp cDNA that encodes human myeloid proteolysis protein (PLP) and have used this clone to examine PLP mRNA expression in developing human spinal cord between 11 and 24 weeks after conception. Fetal tissues were obtained within minutes of elective abortions, frozen immediately in liquid nitrogen, and stored at −80°C.

Poly(A)* RNA isolated from pooled human spinal cord obtained at three periods of development: 11-14 weeks, 17-19 weeks, and 21-23 weeks. Northern blots containing equal amounts of RNA were examined with a fragment of the PLP cDNA that contained the entire PLP coding region and 450 bp of the 3'-untranslated region. Several size classes of PLP mRNAs were detected, the major bands being at 3.2, 2.7 and 2.3 kb with minor bands at 1.95, 1.5 and 1.2 kb. The abundance of the 3.2 kb RNA increased with age. Densitometric analysis indicated that, relative to the 11-14 week group, the 3.2 kb band was enriched 2- fold in the 17-19 week age group and 20-fold in the 21-23 week age group, at which point it represented 98% of the total integrated area of the PLP-hybridizing bands. In the 11-16 and 17-19 week age groups, the 3.2 kb band represented 36% and 12%, respectively, because of the complex expression of the smaller mRNAs. The PLP-hybridizing band at 2.9 kb also appeared to increase with age but this component was relatively minor. The 2.5 kb band was most prominent in the 17-19 week age group where it represented approximately 70% of the total integrated area of the PLP-hybridizing bands. Examination of PLP mRNAs in total cellular RNA isolated from spinal cord confirmed the age-dependent increase of the 3.2 kb PLP mRNA and indicated that the prominent 2.3 kb band emerged around 16 weeks. Some of these smaller bands may represent RNAs encoding DM-20, a myelin protein closely related to PLP. Other studies suggest that the 3.2 kb PLP mRNA sequence is identical to PLP except for an internal deletion. The smaller RNAs may also arise from the utilization of alternate polyadenylation signals. In the 17-19 week spinal cord of the human, PLP mRNA. Immunoblot analysis of individual spinal cord homogenates indicated that polyproteins for PLP and DM-20 may be detected at 18-21 weeks of conceptional age whereas polyproteins for myelin basic protein were present 3 to 4 weeks earlier.
CARBAMAZEPINE STUDIES ON BRAIN PROTEIN SYNTHESIS. S. Tewari.*

Carbamazepine (CBZ) is an anticonvulsant drug with a structure similar to antidepressants. Although effective in treating manic-depressives, to date the mechanism by which CBZ exerts its antidepressant effects remains unclear. Studies from our laboratories have demonstrated that CBZ increases gene expression and protein synthesis in the brain. 

Two month old male Sprague-Dawley rats were intragastrically given CBZ. Protein extracts were analyzed using nitrocellulose filters containing polyribosomes. In vivo studies were performed at various times after CBZ administration, and in vitro studies were performed with brain polyribosomes isolated by centrifugation through a 5.7 M CsCl step gradient. Data thus support that the stimulatory effect of CBZ on protein synthesis in vivo is due to activation of the polysome chain initiation reaction of translational processes or because of direct availability of endogenous self supporting soluble factors supplemented medium.

We now report that this protein appears to be glycosylated and characterized the axolemma from cerebellar granule cell axons. Granule cells were isolated from the cerebella of 8d rat pups as described by Annunziata et al. (Dev. Brain Res. 8:261, 1983). The cells were maintained for 8d at which time they have elaborated an extensive neuritic outgrowth. The cells were gently homogenized and the proximal fraction was separated on a 15-40% linear sucrose density gradient. Morphological staining on 8d cultures was used in the identification of the perikaryal and neuritic membranes. AChE histochemical reactivity was primarily associated with granule cell perikaryal plasma membranes. Concanavalin A binding combined with immunohistochemical staining was observed predominately along neurites, although appeared on the perikaryal membrane. Using a radiometric assay for AChE activity on the fractionated membranes revealed that the perikaryal, AChE enriched, membranes were maintained for 8d at which time they have elaborated an extensive neuritic outgrowth. The cells were gently homogenized and the proximal fraction was separated on a 15-40% linear sucrose density gradient.

While numerous studies have shown that the signal inducing gliogenesis provides a myelination cue for the neuron, the identity of the factor is unknown. We have previously described the axolemma from myelinated and unmyelinated axons. While the current studies were performed using plastic cultures, we are currently using plastic cultures for comparing the axolemma from cerebellar granule cell axons, which are non-myelinated and unshaved axons. We have observed the presence of GM as the message in the brain at embryonic day 16, the earliest day tested. The message level increases from the low embryonic level to an elevated amount which is maintained through adulthood. Since GM has been shown to be contained in glial cells in the CNS, we can examine the distribution of GM mRNA in corticospinal and corticaleurons. We have recently identified the axolemma from cerebellar granule cell axons in chemically-defined medium. Our results from both northern analysis and gel shift hybridization indicate that GM, though present at low levels in neurons, is principally located in astrocytes. Through the use of mixed cultures we can assess the interactions between glial and neuronal cell types that may be expected if astrocytic GM were playing an important role in regulating neurotransmitter glutamate.


Glutamine synthetase is involved in the uptake and regulation of glutamate and GABA in the CNS. A major mechanism for the inactivation of transmitter is a high affinity uptake into the astrocyte. The glutamate taken up in this manner is converted to glutamine, which is then freely available to the adjacent neuron as a transmitter process. The enzyme that catalyzes the conversion of glutamate to glutamine (GS) is of great interest. Our aim has been to investigate interactions between gial cells and neurons which might be involved in the developmental regulation of this enzyme.

To this end we have cloned a 2.5 Kb rat brain cDNA fragment from a lambda gill library through complementarity to a hamster genomic clone of GS. This clone recognizes a 3 Kb sense strand on Northern analysis, which is the approximate size seen in both hamster and avian cells.

Partial sequencing of this clone shows a high degree of homology to the published hamster GS cDNA sequence.

The whole brain, brain regions, and various body tissues from rats ranging in age from embryonic day 16 to adult were homogenized in guanidinium isothiocyanate, and the RNA purified by centrifugation through a 5.7 M CsCl step gradient. Equal quantities of total RNA, as determined spectrophotometrically, were run on denaturing formaldehyde gels and transferred to nitrocellulose. Hybridizations were carried out with 32-P nick-translated and cDNA probes to GS. The GS probe detected a similarly sized message in all tissues examined, with the level of GS message being highest in the brain, followed by kidney, liver, skeletal muscle and heart in decreasing order. We have observed the presence of GS message in the brain at embryonic day 16, the earliest day tested. The message level increases from the low embryonic level to an elevated amount which is maintained through adulthood.

Since GS has been shown to be contained in glial cells in the CNS, we can examine the distribution of GS mRNA in cortical and cerebellar astrocytes and cerebellar granule cell axons. We have recently identified the axolemma from cerebellar granule cell axons, which are non-myelinated and unshaved axons, in chemically-defined medium. Our results from both northern analysis and gel shift hybridization indicate that GS, though present at low levels in neurons, is principally located in astrocytes. Through the use of mixed cultures we can assess the interactions between glial and neuronal cell types that would be expected if astrocytic GS were playing an important role in regulating neurotransmitter glutamate.
473.4 CHANGES IN PHOSPHATIDYLserine CONTENT ALTER PROTEIN KINASE C ACTIVITY IN A HUMAN NEURONAL CELL LINE. L.E. Slack*, M. Litcovitch, E. Rehovot, Israel.

Phosphatidylserine (PS), an important, negatively charged phospholipid component of mammalian cell membranes, can potentially influence cell function because of its ability to alter the physical characteristics of lipid bilayers and to modulate the activity of certain membrane-bound enzymes. We have begun to study the relationship of PS content to the physiological and biochemical characteristics of a human neuronal cell line (LA-N-2). In this system it is possible to manipulate phosphatidylserine content. When exposed to liposomal suspensions of PS, the transmural ion fluxes of these periods of exposure. LA-N-2 cells showed a time and concentration-dependent increase in cell PS content. Incubation of the cells with 25 μM PS in the medium, the PS content of the cell lysate rose within 4 hours, from 5.9 ± 1.2% of total cell phospholipids to 28.3 ± 3.7% (mean ± SEM), and further to 38 ± 1.6% after 24 hours. Lower concentrations of PS were present in the medium, the cell PS content increased progressively over six days (the longest time period studied). Cells treated with PS showed a pronounced configuration, withdrawing the neurites which are normally extended in serum-free medium. Electron micrographs of the PS-treated cells showed vacuoles in the cytoplasm which were absent in controls. These may represent sites of storage for the large amounts of PS in the cells. However, at least some of the PS taken up by the cells was also incorporated into cellular membranes. In preliminary experiments in which cells were lysed and subcellular membrane fractions were prepared by centrifugation on a sucrose density gradient, increases of several-fold in the ratio of PS to total phospholipids were measured in the membranes from cells treated to PS for 24 hours. In parallel experiments, changes in the activity of the PS-dependent enzyme, CA/Pkinase, were measured on untreated (LA-N-2) cells. In untreated LA-N-2 cells, PKC was translocated from the cytosol to the membrane in a concentration-dependent manner by the transmural treatment, this translocation was enhanced by PS treatment (P). This response was accompanied by a rounding of cell morphology similar to that noted in cells stimulated with phorbol ester. A concentration of 25% of total phospholipids) the concentration of PKC required to produce a half-maximal translocation of PKC was increased five-fold. PKC- and PS-induced stimulation of PKC was evident in the response curve. These results demonstrate that alterations in membrane PS content in LA-N-2 neuroblastoma cells affect the activity of PKC. This transmural medium influences cell function known to be regulated by PKC, including differentiation, proliferation, and transmitter release.

473.6 PURIFICATION OF A CALSEQUESTRIN-LIKE PROTEIN FROM BOVINE BRAIN R.E. Berson*, R.J. Fink*, Iowa City, IA.

Calsequestrin (Cq), a cation-chelating protein, is localized in the sarcoplasmic reticulum of skeletal muscle and has many functional similarities to a cationic acidic proteins, such as the Cq from skeletal muscle. In this study we have isolated a cationic acidic protein from biological extracts of rat brain that shows a high degree of homology with the cationic acidic proteins. The Cq has a high degree of homology with the cationic acidic proteins.
473.7 EFFECTS OF MELATONIN TREATMENT ON PHOSPHOLIPIDS IN THE MEDIAL BASAL HYPOTHALAMUS (MBH) AND CORTEX OF HAMSTERS. K. Rud even, L. Potter berg, R. Chandrasekhar, M. Navidi and G. Sun. Departments of Anatomy and Biochemistry, University of Missouri School of Medicine, Columbia, Missouri.

Chronic melatonin administration to female hamsters leads to acyclicity and gonadal atrophy. The specific mechanism by which melatonin acts upon the hypothalamic-gonadal axis is unknown. The effects of long term melatonin administration on the incorporation of 32Pi into MBH polyphosphoinositides and membrane phospholipid composition was examined in this study. Adult female Syrian hamsters were given daily afternoon injections of melatonin (25ug) or ethanol-saline vehicle for 10 weeks. In the first experiment, 12 melatonin-treated and 12 vehicle-treated animals were given 32Pi into the third ventricle either early or late in the light period of the photoperiodic cycle. All animals received 32Pi 12 h prior to the lights off. The MBH was excised and processed for incorporation of 32Pi into phospholipids. In the second experiment, 4 melatonin-treated and 4 vehicle-treated hamsters were given 32Pi intraventricularly late in the light phase and killed 14 h later. Incorporation of 32Pi into polyphosphoinositides (PI's) was measured in the MBH and cortex of the brains. The results from the first experiment indicate the presence of a diurnal rhythm in the incorporation of 32Pi into phospholipids. There was an increase in labelling of PC in animals given the 32Pi late in the photophase compared to those injected with 32Pi early in the daytime. The diurnal variation was more apparent in melatonin-treated animals where 32Pi incorporation into PC and SPH was increased (p < 0.05) during the nighttime more than during the daytime. Also, PE and DPE were reduced in the nighttime when compared to daytime levels in melatonin-treated animals. The phospholipid ratios of PS/PI, PS/PE and PC/PE were significantly increased (p<0.05) during the nighttime in melatonin treated animals. In the second experiment, 32Pi incorporation into polyPI's was significantly increased in the MBH of melatonin-treated hamsters compared to vehicle-treated animals (p < 0.025). PIP-2 and PIP were increased in melatonin-treated hamsters, by 15% and 16% respectively. 32Pi incorporation was not significantly altered in the cortex of melatonin-treated animals. These data suggest that melatonin may alter membrane phospholipid composition and 32Pi incorporation into polyphosphoinositides. The action of melatonin on MBH phospholipids may be related to a possible mechanism whereby melatonin acts upon the reproductive system by altering phospholipid metabolism in the MBH. (Supported in part by NS20836 to G. Sun and HD17658 to L. Peterberg.)


Phosphotidyl choline (C), phosphatidyl ethanolamine (E), phosphotidyl serine (S), sphingosylphosphorylcholine (P) represent the five main phospholipids of brain membrane. We have recently shown that the phospholipid profile of brain membrane can be readily studied using 31P nuclear magnetic resonance (NMR) spectroscopy of conventional brain lipid extracts. Although this technique is not directly applicable to live patients, autopsy or biopsy material can be analyzed promptly and accurately.

The following figures are representative 31P spectra of normal and cerebral cortex, subcortical white matter, corpus callosum, and thalamus. Unique phospholipid profile consistent with known profiles based on conventional biochemical assays for each region is apparent.

Supported by grants from the NIH (GM 37197, RR 02479) and the Veterans Administration Research Service.

473.9 CHOLESTEROL CONTENT AND FLUID PROPERTIES OF SKELETAL MUSCLE SARCROLEMMAL MEMBRANE VESICLES IN MATURE ADULT AND AGED RATS. K.D. Williams and D.O. Smith. Dept. of Physiology, University of Wisconsin, Madison, WI 53706.

In previous research designed to uncover potential changes in membrane lipid composition that could explain age-related increases in permeability and turnover of free choline we evaluated the effects of aging on the composition of skeletal muscle membrane lipids. A rise in plantaris, gastrocnemius, and diaphragm cholesterol levels of about 15% was observed between the ages of 10 and 25 mos in male Fischer 344 rats. Since sarcolemma contains a high fraction of cholesterol, we sought to determine whether this age-related increase was localized in the sarcolemma. A related aim was to establish whether the sarcolemmal fluidity was affected by aging.

Hindlimb muscle (20-30g) was obtained from rats anesthetized with chloral hydrate and homogenized in sucrose buffer. Crude membrane containing sarcolemma from the broken fibers was extracted with LiBr buffer and purified further by washing in KCl buffer and sucrose gradient centrifugation. The sarcolemmal fraction obtained at the interface between 15 and 28.5% (w/v) sucrose did not differ in levels of cholesterol (317 and 332 nmol/mg protein) or phospholipid (879 and 871 nmol/mg protein), or their molar ratio (0.362 and 0.386) between rats aged 10 and 25 mos, respectively.

To assay membrane fluidity, the activation energies for membrane lipid composition that could explain age-related increases in permeability and turnover of free choline we evaluated the effects of aging on the composition of skeletal muscle membrane lipids. A rise in plantaris, gastrocnemius, and diaphragm cholesterol levels of about 15% was observed between the ages of 10 and 25 mos in male Fischer 344 rats. Since sarcolemma contains a high fraction of cholesterol, we sought to determine whether this age-related increase was localized in the sarcolemma. A related aim was to establish whether the sarcolemmal fluidity was affected by aging.

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To assay membrane fluidity, the activation energies for

membrane lipid composition that could explain age-related increases in permeability and turnover of free choline we evaluated the effects of aging on the composition of skeletal muscle membrane lipids. A rise in plantaris, gastrocnemius, and diaphragm cholesterol levels of about 15% was observed between the ages of 10 and 25 mos in male Fischer 344 rats. Since sarcolemma contains a high fraction of cholesterol, we sought to determine whether this age-related increase was localized in the sarcolemma. A related aim was to establish whether the sarcolemmal fluidity was affected by aging.

Hindlimb muscle (20-30g) was obtained from rats anesthetized with chloral hydrate and homogenized in sucrose buffer. Crude membrane containing sarcolemma from the broken fibers was extracted with LiBr buffer and purified further by washing in KCl buffer and sucrose gradient centrifugation. The sarcolemmal fraction obtained at the interface between 15 and 28.5% (w/v) sucrose did not differ in levels of cholesterol (317 and 332 nmol/mg protein) or phospholipid (879 and 871 nmol/mg protein), or their molar ratio (0.362 and 0.386) between rats aged 10 and 25 mos, respectively.

To assay membrane fluidity, the activation energies for
474.1 INTRAHIPPOCAMPAL INJECTIONS OF GALLAMINE IMPAIR LEARNING OF A REPRESENTATIONAL MEMORY TASK. W.S. Messer, Jr. and M.D. Miller.* Department of Medicinal Chemistry, College of Pharmacy, University of Toledo, Toledo OH 43606.

Recent studies indicate that gallamine binds selectively to M-like muscarinic receptors (Buhe, *J Pharmacol*) 30: 56; 1960; Price et al., *Biochem Pharmacol* 38: 4171, 1986) which may be presynaptic receptors in forebrain that regulate cholinergic activity associated with memory, rats were trained to produce signs of cholinergic stimulation including salivation, piloerection and tremors in addition to induction seizures (Messer et al., *Exp Proc* 46: 857, 1987).

To determine whether preynaptic cholinergic antagonists can be utilized to potentiate cholinergic activity associated with memory, rats were trained to perform a matching to sample memory task in a T-maze. Young male Long-Evans rats were implanted stereotaxically with cannulae aimed at each hippocampus (AP, -4.6; ML, ± 3.7; DV, -4.0). Following surgery, rats were maintained at 85% of ad lib weight and trained to move through the maze for food over a period of two weeks prior to learning the memory task.

Once animals responded within three seconds on one-door runs, they were ready for injections and subsequent learning of the win-stay memory task. Animals were pressure-injected with either saline or 10 μg of gallamine triethiodide in 1 μl of saline bilaterally (5 μg to each side) 10 to 30 minutes before each testing session. Animals were given two runs per trial with a 30 second delay between each run. The first (information) run to a single arm was rewarded with food, while the second (choice) run was rewarded only if the animal chose the arm entered previously (win-stay or match-to-sample).

Saline-injected animals (N=4) learned the task slowly and achieved 100 % as a group on the second run. (Block of twelve trials). (Saline-injected animals, N=5) achieved a higher percentage of correct choices on the first exposure to galantamine, but the difference between the two groups was not significant. By the eighth session, galamine-injected animals were performing below the level attained by saline-injected animals. Galamine-injected animals as a group were able to achieve only 10-20% correct choices on the last session.

The difference between the two groups was statistically significant over the last four sessions (p < 0.025 as determined by χ² analyses). The data suggest that although galamine increases acetylcholine release, adaptive mechanisms (such as receptor down-regulation) may prevent an enhancement of memory. This work was supported by a grant from the Ohio Board of Regents.


Cholinergic neurons function in several different brain systems. Those neurons in the nucleus basalis of Meynert, are highly affected in Alzheimer’s Disease (AD) while others in the basal ganglia are affected in schizophrenia. The existence of neurons using the same neurotransmitter but anatomically isolated in ventral and dorsal portions of the basal ganglia, each relationship each neuron has with its own surround made it difficult to explore the pharmacological nature of neurons using acetylcholine. Yet, the role of cholinergic activity is of major importance in the treatment of these diseases, and the effects of antimuscarinic agents to extend this line of study.

To further elucidate the behavioral effects of antimuscarinic agents as a potential model of AD, we administered scopolamine to 3 elderly monkeys (>21 years) performing a choice reaction task. Choice reaction is slowed in AD patients. In the task, a red or green TV screen was presented, lower green up or down, respectively, was rewarded with ic juice, 320 trials/2 hours. Scopolamine was administered (doses of 10, 20, 15, 5, 25 μg/kg IM) once per week to each monkey (one, two, and three). Inactive placebo (saline) and active placebo (glycopyrrolate) this drug has about twice the peripheral potency of scopolamine in humans, therefore, we used 1/2 scopolamine dose) was given on the other days (drug administrated 6 min before testing).

For saline, the average responsiveness was 84% ±11, correctness was 89% ±6. At low doses, scopolamine slightly impaired response/veries (85 μg/kg: avg=54) but not correctness (85 μg/kg: avg=50). At the next dose (25 μg/kg: avg=58), reaction time was slightly improved (see table). However, at higher doses, scopolamine caused a major decrease of animal responsiveness (80 μg/kg: avg=42) and correctness (25 μg/kg: avg=72). Only at the highest dose was reaction time slowed below the saline mean (see table): AVERAGE RESPONSE/VERIES PER TESTING SESSION (new):

<table>
<thead>
<tr>
<th>Dose (μg/kg)</th>
<th>Response/Veries (avg)</th>
<th>Correctness (avg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>84% ±11</td>
<td>89% ±6</td>
</tr>
<tr>
<td>10</td>
<td>85% ±6</td>
<td>85% ±6</td>
</tr>
<tr>
<td>20</td>
<td>85% ±6</td>
<td>85% ±6</td>
</tr>
<tr>
<td>25</td>
<td>85% ±6</td>
<td>85% ±6</td>
</tr>
<tr>
<td>50</td>
<td>85% ±6</td>
<td>85% ±6</td>
</tr>
</tbody>
</table>

Response/veries was significantly slower than saline on the highest dose, but the difference was not statistically significant. The data suggest a consistent effect on performance, though it had a tendency to impair correctness at the highest doses and delayed effects were suspected.

Now do scopolamine may be affecting motor function by affecting the basal ganglia as such drugs benefit Parkinson patients (a neurological disorder of schizophrenia). However, the behavioral disruption at the higher dose is similar to that seen with AD patients performing related tasks.


Baclofen (5-ethylphenethyl-GABA) is an antispastic drug known for its relative absence of side-effects, even at high doses. It has been suggested that baclofen, when given in combination with drugs having anticholinergic properties, may produce a state of confusion and possible memory deficits greater than those seen with anticholinergic drugs alone. To test this hypothesis, 16 male Fischer-344 rats were trained on an eight-arm radial maze for food reinforcement. The effects of various doses of baclofen (1.25 or 2.5 mg/kg) and scopolamine (0.1875, 0.375, and 0.75 mg/kg), alone and in combination were determined. Baclofen alone did not significantly affect measures of cognitive function (number of correct responses in the first eight responses, total errors), or the time required to complete the maze. Scopolamine alone decreased the number of correct responses in the first eight choices, while increasing both the number of errors and the time necessary to complete the maze. A subjectively-noted increase in circling behavior and ataxical responses was also present in scopolamine-treated rats. When the two drugs were co-administered, baclofen had no effect on the number of errors or time required to complete the maze in the presence of scopolamine; however, in conjunction with the high dose of scopolamine, it significantly potentiated the decrease in number of correct responses. In the first eight choices. In a subsequent experiment, the interactive effects of baclofen and scopolamine on motor activity were examined. Baclofen exhibited no dose-response effect on motor activity, whereas all doses of scopolamine increased activity. The higher dose of baclofen attenuated scopolamine-induced hyperactivity by 50%, but the lower dose of baclofen was not effective. These data support the conclusion that baclofen exacerbates behavioral deficits produced by drugs with anticholinergic properties. In the radial-arm maze, yet antagonizes the hyperactivity seen with these drugs in motor activity chambers. (This work supported in part by NSF grant R00561791.)

474.4 EFFECTS OF THE ANTICHLORINESTERASE PARAOXON AND THE MUSCARINIC ANTAGONIST ATROPINE ON RETENTION OF LEARNED BEHAVIOR AND ON STRIATAL MUSCARINIC RECEPTORS. J.K. Chambers and R.V. Chambers*. Deps. of Biological Sciences and Entomology, Mississippi State University, Mississippi State, MS 37962.

Cholinergic hyperactivity and hypoactivity would be induced following administration of an acute treatment of an anticholinesterase or an acetylcholinesterase antagonist, respectively. In the case of a persistent organophosphate anticholinesterase, chemical lesioning with a number of days following treatment. Acute high level exposures to paraaxon (P; diethyl phosphorothioate, active metabolite of the insecticide parathion), and two forms of atropine (atropine sulfate, AS, active centrally; and atropine methyl bromide, ABB, inactive centrally) alone or in combination, were studied for their effects on the retention of fixed ratio performance and on muscarinic receptors in the corpus striatum of male rats. High doses were given to mimic a severe accidental poisoning. Adult male rats (Rattus norvegicus), Sprague Dawley derived strain, which were food deprived, were trained to a fixed ratio 10 schedule of food rewarding for food pellet reinforcement. Following stabilization, they were injected intraperitoneally with the drugs or vehicle and were tested for retention of FR10 performance for up to 4 days following treatment.

Animals were sacrificed at 1,2 or 4 days following treatment for assay of [3H]quinuclidinyl benzilate binding to muscarinic receptors and for acetylcholinesterase activity. Performance was greatly depressed by AS and ABB and AS+P 1 day after treatment with gradual recovery. Significant differences were observed after exposure to P alone at 1 day with recovery by 2 days. Performance was unaffected by AS. The percent inhibition of brain acetylcholinesterase observed at 1,2 and 4 days after treatment with P was about 85%, 65% and 45%, respectively. Small increases in muscarinic receptor numbers at 1 day after treatment with atropine and decreases were observed 4 days after treatment with P. The changes in fixed ratio performance following drug treatment are considerably more dramatic than the changes observed in striatal muscarinic receptors. (Supported by EPA grant B-811295).
**474.5 EFFECTS OF NEUROACTIVE AGENTS ADMINISTERED INTRACRANially ON BEHAVIORAL INHIBITION IN CATS.** M.M. Curtis*. P.M. Saxton (Neurology and Neurosurgery, Institute of Neuroscience and School of Life and Health Sciences, Univ. Delaware, Newark, DE 19716).

In a series of experiments, CNS inhibitory mechanisms were investigated using a differential reinforcement of low rate of response (DRL) operant task. Six cats were trained on the DRL task which involves an active behavioral inhibitory process. Cannulae were implanted in the basolateral amygdaloid and medial septal nuclei for the administration of serotonin (5-HT)(45 μg), fluoxetine (16 μg), L-norepinephrine bitartrate (NE)(45 μg), or acetylcholine (ACh)(60 μg). All drugs were administered in a 1.0 μl suspension. Control conditions involved injections of vehicle and performance on a noninhibitory task (a fixed rate schedule).

Performance indices (errors, reinforcements, and inter-response times [IRTs]) were tabulated for each 3 min period in a 30 min test session. Errors on the DRL schedule were made when an animal failed to withhold a bar-press for a specified period of delay time (e.g., 15 sec). Reinforcement (liquid mash) was received when an animal bar-pressed at the correct time.

Fluoxetine, which elevates serotonin levels, improved performance on the DRL (errors decreased; reinforcements increased) when injected into the amygdala and septum. 5-HT into both brain sites reduced errors and reinforcements through an overall slowing in bar-press response rates (IRTs increased). Both NE and ACh impaired DRL performance (errors increased; reinforcements decreased) when injected into the septum. Both NE and ACh into the amygdala caused a slowing in response rates.

These experimental results indicate that there is a complex interaction of several neurotransmitter systems (5-HT, NE, and ACh) in the amygdala that appears to exert a facilitatory influence on a behavioral inhibitory system. CNS inhibitory mechanisms are greatly impaired when NE levels are elevated in the septum. Other agents (e.g., clomipramine) will be tested prior to histological verification of cannula placement.

**474.6 GLUCOSE AND EPINEPHRINE ATTENUATION OF SCOPOLAMINE-INDUCED LOCOTOR ACTIVITY IN MICE.** W.S. Stone, K.L. Cottrill* and P.E. Gold. Dept. Psychology, University of Virginia, Charlottesville, VA 22901.

Many species exhibit impaired cholinergic function in old age, with prominent deterioration of cholinergic systems observed in various age-related disorders, including Alzheimer's Disease (AD). In addition, the presence of age-related declines in memory and learning have also been associated with both normal and pathological aging processes. We have previously observed that epinephrine (EPI), which increases hepatic glucose (GLU) among other physiological and psychological responses, can store memory in aged male mice (Sternberg et al., Beh. Neurol. Biol. 1986). Scopolamine (COMPOUND B BM-5 (N-methyl-N-(1-methyl-4-pyrrolidin-2-butyryl)-acetyl) antagonizes as a model of aged memory impairment. Recently, we found that SCOP-induced amnesia can be partially attenuated by posttraining (EPI) and (GLU), but not scopolamine (ALZ-134) in young mice. The dose-response curve was an inverted-U with maximal attenuation at 0.05 mg/kg with EPI and 100 mg/kg with GLU. In the present experiment, we explored the possibility that hyperactivity produced by SCOP could also be attenuated by EPI or GLU injections.

Activity levels, measured by the number of lines crossed in an acrylic compartment, were assessed for 30 minutes, starting 50 minutes after male mice (BNBR-ICR) were injected with SCOP (3.0 mg/kg), meth-SCOP (3.0 mg/kg) or saline (5% SAL). Only SCOP increased activity levels above those of SAL, suggesting that the effect was produced centrally rather than peripherally.

Additional groups of mice were then injected with EPI, GLU, AREC, or physostigmine (PHYSO), administered 30 min after the SCOP (20 minutes before assessment activity levels). The results of both EPI and GLU significantly attenuated SCOP induced activity in an inverted-U dose dependent manner, with maximally effective doses comparable to those observed in memory tests. Of the cholinergic agonists, PHYSO (0.2 mg/kg) but not AREC (10 mg/kg) significantly attenuated the hyperactivity. In addition, a combination of GLU and PHYSO produced significantly greater attenuation than did either drug alone.

These results indicate that EPI and GLU can reduce the effects of cholinergic blockade induced by SCOP as measured behaviorally using either memory or hyperactivity tests. We also suggest that GLU may interact synergistically with cholinergic agonists to attenuate effects of cholinergic impairments seen during aging. The anti-impairment by non-cholinergic agonists suggests the importance of interacting neural systems for an understanding of both age-related changes and their possible amelioration. [Supported by the Office of Naval Research (N00014-85-K0472) and the American Diabetes Association.]

**474.7 DISCRIMINATIVE STIMULUS PROPERTIES OF PHYSOSTIGMINE IN RATS.** S.R. Franklin* and A.H. Tang, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

The cholinesterase inhibitor, physostigmine (PHY), has been shown to improve memory functions in both normal human subjects and in Alzheimer's patients (Davis et al., Science 201:772, 1978; Davis and Mohs, Am. J. Psychiatry, 139:147, 1982). Experimental subjects have reported characteristic subjective effects (e.g., nausea, slowed thoughts, mild sedation, etc.) produced by PHY (Davis et al., Psychopharmacol. 51:24, 1976). In this context, the differential effects of PHY have been reported as subcutaneous injection of PHY (0.2 mg/kg) from a similar injection of saline in a two-lever, food-reinforced behavioral paradigm. These results indicate the dose of PHY reduced the response rate to about 50% of that in saline sessions. The discriminative stimulus (DS) effect of PHY at the training dose is mediated by a muscarinic mechanism in the CNS, since it was antagonized by scopolamine (0.1-0.3 mg/kg), but was unaffected by methyl-scopolamine (1 mg/kg) or pirenzepine (3 mg/kg), which do not enter the brain readily. The dose-reducing effect of PHY was not affected by the above antagonists. The peripherally-active cholinesterase inhibitor, neostigmine, produced predominantly saline-appropriate lever choice at doses that did not completely suppress responding. Compounds which produced average reductions of greater than 80% responses on the PHY lever are Compound BM-5 (N-methyl-N-(1-methyl-4-pyrrolidino-2-butyryl)-acetic amide), THA (1,1-d,3-acetate-2-chloro-5-nicotine), RS-86 (2-ethyl-3-methyl-2,4-diazoxazol-5-(4)-dec-1-on, hydrobromide), and pirenzepine. In comparison, oxotremorine, arecoline, and acetylcholine (3-acetoxyquinuclidine) produced a maximum average reduction of 40-60% on the PHY lever before the behavior was completely abolished. The DS effect of PHY in rats may be related preferentially to activation of the M1 muscarinic receptor in brain.


Exercise has been shown to influence behavioral sensitivity to a number of compounds acting primarily on the cholinergic system. Acute exercise appears to increase sensitivity, whereas a decrease in sensitivity can be demonstrated in response to chronic exercises. The effects of acute exercise on behavioral sensitivity to physostigmine (PHYSO), as well as the effect of acutely administered PHYSO on ability to exercise, have been evaluated in rats. In a separate study, non-muscular examination of muscle tissue from drug-challenged, exercised animals has been completed.

**Experiment 1.** Rats trained to lever press for food reward under a fixed ratio (FR50) schedule of reinforcement completed a 20-week exercise conditioning program. No exercise conditions, trained on the F0 task, served as control subjects. When rested, the control animals displayed a typical dose-related performance decrement (15, 46, 73, and 69%) in response to four doses of PHYSO (50, 100, 150 and 200 g/kg). Rats that had completed the exercise-conditioning program continued to perform at near-normal levels over the same dose range. When a 15-min treadmill session preceded PHY administration, F50 performance remained above 60% of baseline levels in the exercise-conditioned group across all doses. In contrast, responding by control animals fell to below 20% of baseline performance at the lowest dose studied, 75 ug/kg, is below the threshold for FR30 behavioral effects, produces 66% cholinesterase (ChE) inhibition in whole blood, and does not induce neuromuscular lesions in sedentary rats. When administered 15 min prior to exercise, this dose prevented completion of a standard exercise session. Lower dose studied, 7.5 ug/kg, produced 30% inhibition of whole blood ChE and no apparent difficulty in completion of a 30-min exercise session. Two doses of drug administration, animals were anesthetized with sodium pentobarbitol and vascularly perfused with saline followed by glutaraldehyde fixation. Dissections, sensory organs, and other structures were dissected and processed for histological localization of ChE and other enzymes (CH). Small pieces of muscle containing stained NMJs were further processed for standard electron microscopy. Analysis of thin sections from the high dose group revealed occasional myofibrillar damage but little evidence of extensive NMJ damage. 

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474.9 EFFECTS OF NICOTINE AND CANNABINOLS ON EXTRAPYRAMIDAL MOTOR BEHAVIORS. P.R. Menderscheid, E.D. Moss, N. Kobayashi*, and S.P. Montgomery. Department of Psychology, Univ. of Texas at El Paso, El Paso, TX 79968.

Although delta-9-tetrahydrocannabinol (THC) and related cannabinoids have been studied extensively for several years, the biochemical mechanism(s) by which THC alters physiological and behavioral function is still unknown. Our working hypothesis of our laboratory is that the cannabinoids are acting as nicotinic cholinergic agonists within the extrapyramidal system to potentiate hypokinesia induced by direct or indirect dopamine antagonists.

The conditions under which extrapyramidal effects of cannabinoids and nicotine can be observed are relatively specific: the most important being the blockade of dopaminergic function within the striatum (e.g., after reserpine (RES)-, L-dihydroxybenzene (DLH)-, or haloperidol-induced hypotonia) (Moss et al., 1984).

Our nicotinic hypothesis of cannabinoid action has been derived from experiments which show that (1) THC or a combination of THC and RES does not potentiate hypokinesia (induced by the dopamine antagonist levo-nantradol) in a manner exactly parallel to levonantradol, a specific synthetic agonist of the norepinephrine receptors; and (2) THC/RES hypokinesia is completely reversed by yohimbine, an anticholinergic antagonist (Moss et al., 1984).

Montgomery et al. (1985) have shown that intracranial injections of (-)-nicotine into the caudate-putamen (CP) of reserpine-rat potentiate hypokinesia (as measured by the bar test) in a manner exactly parallel to levonantradol, a specific synthetic agonist of the norepinephrine receptors. In a note of the nicotinic hypothesis, hypokinesia (measured by the motor activity), a non-selective nicotinic cholinergic antagonist, when injected into the CP of reserpine-pretreated rats. We believe that this is an artifact that can be eliminated by using a lower dose of RES.

If the nicotinic hypothesis is correct, other pharmacologically consistent interactions with known nicotinic agonists and antagonists are expected. However, showing that nicotine and cannabinoids are working as nicotinic agonists will be difficult to prove if the cannabinoid-mediated hypokinesia involves a mechanism by which cannabinoids act via different neurophysiological substrates. In order to prove that cannabinoids act via different neurophysiological substrates, receptor binding experiments need to be conducted to determine if the behavioral effects obtained with nicotine and the cannabinoids can be explained by a direct action on central nervous system nicotinic receptors. If our nicotinic hypothesis of cannabinoid action is correct, it must be recognized that this may still be only one of several possible cannabinoid actions.

474.11 A DOSE-RESPONSE CURVE FOR NICOTINE-INDUCED FLAVOR AVERSION LEARNING IN RATS. T.A. Landrum*, D.G. Gilbert*, and R.A. Jensen. Biopsychology Laboratory, Department of Psychology, Southern Illinois University, Carbondale, IL 62901.

The release of opioid neuromodulators in response to nicotine administration may be related to the development of tolerance and dependence. Some of the central effects of nicotine are suggested to be mediated via the nicotinic receptors located in the corpus striatum. In most studies high doses of nicotine were administered to animals and that large amounts of nicotine would be released in animal and human subjects. However, some of these studies may have employed too high doses of nicotine which may in fact potentiate the behavioral response. Therefore, it is important to determine a critical dose of nicotine above which possible confounding transcius is produced. One method of determining whether a given dose of nicotine can produce significant results in rats is in the use of the taste-aversion-learning techniques.

Male Sprague-Dawley rats were maintained on a 20-mg-per-day schedule of water deprivation for 5 days prior to training on a taste-aversion learning task. Using a two-bottle procedure, the rats were given a 10-mg-per-day dose of nicotine, 0.1% saccharin solution on the training day. Ten minutes after drinking was completed, animals received (i.p.) one of six logarithmically spaced doses of nicotine, 0.022, 0.045, 0.10, 0.22, 0.46 mg/kg, nicotine, or 2.0 ml of 0.47 molar lithium chloride. On the test day, 72 hours after conditioning, each rat was given a preference test in which one bottle contained the 0.1% saccharin solution and the other contained distilled water.

Behavioral results obtained in the rats which were trained are seen in the table below. The critical dose for nicotine-induced taste aversion was 0.022 mg/kg.

474.12 ACUTE AND CHRONIC EFFECTS OF NICOTINE ON RAT STRIATAL NICOTINIC AND Dopaminergic SYSTEMS. Y.K. Fung and Y.S. Lee. Dept. of Oral Biology, University of Nebraska Medical Center, College of Dentistry Lincoln, NE 68583 and Dept. of Pharmacology, School of Medicine, Creighton University, Omaha, NE 68178.

Some of the central effects of nicotine are suggested to be mediated via the nicotinic receptors located in the corpus striatum. In most studies high doses of nicotine were administered to animals and that large amounts of nicotine would be released in animal and human subjects. However, some of these studies may have employed too high doses of nicotine which may in fact potentiate the behavioral response. Therefore, it is important to determine a critical dose of nicotine above which possible confounding transcius is produced. One method of determining whether a given dose of nicotine can produce significant results in rats is in the use of the taste-aversion-learning techniques.

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Behavioral results obtained in the rats which were trained are seen in the table below. The critical dose for nicotine-induced taste aversion was 0.022 mg/kg.


A major focus of our laboratory is the identification of genetic factors that regulate behavioral and physiological responses to acute nicotine administration. We have demonstrated that nicotine-induced hyperactivity is regulated by at least two independent loci. Our recent results suggest that the dopamine D1 receptor mediates the effect of nicotine on locomotor activity. We have also demonstrated that nicotine-induced hyperactivity, as measured by the open field test, is reduced in adrenalectomized mice. We have found, however, that the strain difference in nicotine sensitivity is not altered in adrenalectomized mice. Our results support the hypothesis that the strain difference in nicotine sensitivity is not altered in adrenalectomized mice.

The release of opioid neuromodulators in response to nicotine injections has a role in the body temperature. Nicotine elicited a dose-dependent increase in heart rate and body temperature. ADX mice of the C57 (control) and DBA/2J (nicotinic response) strains were more sensitive than sham operated controls in all parameters tested.

This research was supported by a grant from the R.J. Reynolds Tobacco Company to R.A.J. and D.G.G.
474.13 EFFECTS OF CARBAMATE PRETREATMENT AND OXIME THERAPY ON SOMAN-INDUCED PERFORMANCE DECREMENTS AND BLOOD CHOLINESTERASE ACTIVITY IN PRIMATES. D. W. Black*, R. B. Murphy*, G. C. Brown*, D. L. Hartweg*, and M. G. Tocchetti*. USDA Sch. Aerosp. Med., Radiat. Sci. Div. Brooks AFB, TX 75235. Performance by well-trained rhesus monkeys of the Primate Equilibrium Platform (PEP) compensatory tracking task after exposure for 30 min before and 90 min after exposure to soman (2.05-2.78 ug/kg) with simultaneous atropine (97 ug/kg) therapy. The ED50 soman dose to produce PEP performance decrement estimated by the up-and-down method was 2.27 ug/kg (95% confidence interval: 2.15 < ED50 < 2.40 ug/kg). Another group of monkeys was pretreated with 150 ug/kg of pyridostigmine bromide (PYR) 30 min before soman exposure and given a therapeutic dose (17 ug/kg) of 2-PAM Chloride (2-PAM) in addition to atropine. This group had an ED50 soman dose of 2.56 ug/kg (95% confidence interval: 2.41 < ED50 < 2.75 ug/kg).

A similar pretreatment-therapy regimen provides substantial protection against the lethal effects of soman exposure. However, the protection against soman-induced performance decrements that we measured (Protection Ratio = 1.4) has little or no practical significance. The control group and the group treated with PYR and 2-PAM had similar levels of serum cholinesterase (ChE) inhibition 24 h and one week later. A separate study of changes in ChE activity after soman exposure showed that 2-PAM therapy reduced ChE inhibition at all times after soman exposure. PYR pretreatment changed the time-course ChE inhibition, producing higher levels of inhibition early (5 h) and lower levels later (26 h) after soman exposure. The 2-PAM and PYR effects combined blunted significantly.

Results for inhibition of ChE activity in whole blood were qualitatively similar to those of the mice except that ChE inhibition in PYR pretreated animals never exceeded the inhibition observed in animals that did not receive PYR pretreatment.


Physostigmine, a short-acting cholinesterase inhibitor, improves memory in normal subjects (Davis, EL et al, Science 201:272-274, 1978) and in Alzheimer's patients (Christie, JE, et al, Brit J Pharmacol 111:464-500, 1993). We have examined the cerebral metabolic effects of physostigmine in 30 ml saline administered intravenously in two consecutive PET studies. Subjects performed a complex reading memory task (CRT) continuously for 30 min after the injection of 18FDG and were scanned for 20 min. Physostigmine infusion was started at the end of the first scan and completed 5 min. after the second 18FDG injection. The second injection was given when CRT was performed again for 30 min and followed by another 20 min scan. CMRglc for the second scan was calculated using a double-injection model validated by us (Chang, et al, J Nucl Med, In Press). CMRglc (mg/100g/min) was 8.91 ± 1.48 before and 8.89 ± 1.16 after physostigmine. No specific regional effects were found. CMRglc scores did not change (35% correct before and 53% correct after physostigmine). These results agree with the observations in rats where physostigmine had no overall effect on CMRglc, but did produce highly localized activation in the superior colliculus and anterior ventral thalamus (Detrich, et al, Ann Neurol 36:117-118, 1984). These results are considered to be compatible with the results of the PET system used. These studies set the stage for similar investigations in Alzheimer's patients.


The purpose of these experiments was to determine the dose-dependent alterations in behavior and hippocampal morphology that follow intradentate administration of colchicine. Male adult Fischer-344 rats received bilateral injections of either 0, 1.25 or 2.5 ug of colchicine into the dorsal and ventral regions of the dentate gyrus. Motor activity of rats was measured over a period of 60 min on three consecutive days. Colchicine produced a dose-dependent increase in both rearing and horizontally directed ambulation. Colchicine-treated rats differed from controls more on the last day of testing than the first day of testing. Light microscopic examination of hippocampal morphology indicated that colchicine produced significant decreases in the length and width of the granule cell lines; the dorsal region of the hippocampus was affected more than the ventral region. Decreases in pyramidal cell length were evident (10-15%), but were generally not dose-dependent. The widths of the CA1 and CA3 regions of the hippocampus were not affected by colchicine and there was no apparent differential sensitivity between the dorsal and ventral hippocampus. Rearing and ambulatory motor activity were statistically correlated with granule cell length and width, particularly for the last day of testing.

Another group of rats was given various doses of colchicine and three weeks later, injected with isotonic saline, s.c., and motor activity was measured. Three weeks later, the rats were challenged with 0.75 mg/kg of scopolamine given s.c. There were dose-dependent increases in motor activity in rats having received colchicine after either saline or scopolamine. However, colchicine-treated rats appeared to be less sensitive to the stimulatory effects of scopolamine than controls. These data are consistent with previous results showing a down-regulation of cholinergic muscarinic receptors in the hippocampus of colchicine-treated animals.

In summary, intradentate administration of colchicine produced a dose-dependent loss of granule cells in the hippocampus, which was highly correlated with increased motor activity and alterations in sensitivity to scopolamine. This is a useful agent to study the functional role of the dentate gyrus and compensatory changes in the hippocampus following lesionsing.


Cannulae were implanted into a forebrain pool of goldfish to determine the effects of site of injection of acetylcholine (ACh) on thermal behavior thermoregulation in goldfish (Carassius auratus; tests: 30-40 °C). Temporal selection was monitored in an aquatic thermal gradient. Positions of fish in this gradient were imaged using a wide angle camera located above the gradient and digitized by an Oculus frame grabber. After an experiment, the gradient was calibrated, and this information was used to convert the stored position values into temperature values. Following 30 min in a thermal gradient, fish were injected with acetylcholine chloride (2-50 ug ACh) in a total volume of 0.2 ul (0.7% NaCl was used as a vehicle). Injections of 10, 25, and 50 ug ACh into the most anterior aspect of the nucleus preopticus periventricularis (NPP; Peter, R.K. & V.E. Gill, J. Comp. Neur., 159:65, 1975) led to consistent, dose-dependent decreases in selected temperature (Tsel). Inconsistent decreases in Tsel were observed following injections of 5 ug ACh into the same neuroanatomical site. No effect on Tsel was observed following injections of 2 ug ACh or control injections. At sites removed from the anterior NPP, no effect on Tsel was observed. Following injections of 25 or 50 ug ACh. Other studies in this laboratory have demonstrated that ethanol and norepinephrine (NE) have thermoregulatory effects when injected into the anterior brainstem. The site of action of ACh was contiguous with that of NE. We conclude the cholinergic inputs to the anterior NPP lead to a decrease in the regulated temperature in goldfish.
VENTRAL PALLIDAL LESIONS PRODUCE DECREASES IN COCAINE AND HEROIN SELF-ADMINISTRATION IN THE RAT. C.B. Ruben*, and G.P. Koob. Scripps Clinic and Research Foundation, La Jolla, CA.

Using the intravenous drug self-administration paradigm, previous work in our laboratory has led to the hypothesis that separate neural systems mediate the reinforcing properties of cocaine and heroin and, furthermore, that these pathways appear to converge at the level of the nucleus accumbens. The results from these previous studies indicated that following ibotenic acid lesions within the region of the nucleus accumbens are important for cocaine reward and that opiate self-administration in rats with lesions in this nucleus is markedly decreased. However, studies in which a separate dopamine system within the nucleus accumbens play a critical role in mediating heroin reward. The ventral pallidum (substantia innominata/lateral preoptic area) is one of the major recipients of nucleus accumbens efferent fibers. The purpose of the present study was to determine the importance of this nucleus accumbens-ventral pallidum pathway in mediating cocaine and heroin reinforcement in the rat.

12 Male rats were trained to self-administer intravenous cocaine (0.75 mg/kg/inj) in 3 hour daily sessions on an FR 5 schedule. After reaching steady baseline responding (± 10% of the average for 3 consecutive days) rats received either bilateral injections of vehicle (N=6) or ibotenic acid (N=6) into the ventral pallidum. When tested 5 days post-lesion, subjects in the lesion group showed a significant decrease in cocaine self-administration when compared to vehicle control animals. A dose-effect determination revealed an overall decrease in responding for cocaine for the lesion group, but the integrity of the typical dose-effect relationship was preserved. The effect of ibotenic acid lesions on heroin self-administration was also assessed using a progressive ratio procedure. In this test, the lesion group displayed a significant decrease in the highest FR value obtained.

The same experimental protocol was used to assess the effects of ibotenic acid lesions of the ventral pallidum on intravenous heroin (0.06 mg/kg/inj) self-administration. Preliminary results indicate that ventral pallidal lesions also produce a significant decrease in heroin self-administration when compared to vehicle control animals.

A decrease in response rate for drug self-administration following neurotoxic lesions is interpreted as an attenuation of the reinforcing properties of the self-administered compound. These results support the hypothesis that the nucleus accumbens project to the ventral pallidum are critical for mediating the reinforcing properties of cocaine and heroin and that the nucleus accumbens-ventral pallidum circuit may be a common second-order link for both stimulant and opiate reward.

VENTRAL PALLIDAL LESIONS PRODUCE DECREASES IN COCAINE AND HEROIN SELF-ADMINISTRATION IN THE RAT. C.B. Ruben*, and G.P. Koob. Scripps Clinic and Research Foundation, La Jolla, CA.
SENSITIZATION TO THE BEHAVIORAL EFFECTS OF COCAINE IS ASSOCIATED WITH ALTERED DOPAMINE METABOLISM AND RELEASE IN RAT BRAIN. Kenneth E. Johnson and Lawrence D. Smith 2 Dept. of Pharmacol. and Toxicol., Univ. of Texas Medical Branch, Galveston, Texas 77550.

The purpose of this study was to test the hypothesis that behavioral sensitization to cocaine is associated with altered dopamine (DA) metabolism in the striatum and nucleus accumbens. In two separate experiments we administered either water or cocaine (15 mg/kg) intraperitoneally for ten days. In the morning of the eleventh day rats were given either cocaine (C) or water (W) and were either killed 15 min later (Exp 1) or rated behaviorally and killed 72 hr later (Exp 2). In either case, the brains were removed and the striatum, nucleus accumbens, medial prefrontal cortex, amygdala, and hippocampus were weighed and homogenized for HPLC-EC determination of DA, DOPAC, HVA, 5-HT, 5-HIAA, and ME. In Exp 2, part of the striatum was cut out into 0.4 mm slices for measurement of DA release. Both experiments consisted of three groups: chronic water-acute water (W-W), chronic cocaine-acute cocaine (C-C), and chronic cocaine-acute water (C-W). Even though behavioral rating only was done in Exp 2, it was evident in both experiments that this regimen produced clear sensitization to cocaine. For example, 15 min post-administration, the group scores were 3.6 ± 0.3 (W-W), 3.6 ± 0.2 (C-C), and 7.5 ± 0.2 (C-W); p<0.05.

In Exp 2, in which the rats were killed 15 min after administration, dopamine metabolism (as assessed by the DOPAC + HVA/DA ratio) was decreased significantly in both the nucleus accumbens and striatum by cocaine in water-treated rats (W-C) relative to control (W-W). However, in cocaine pretreated rats (C-C), cocaine significantly inhibited DA metabolism in the accumbens but not the striatum. This may suggest an alteration in the striatal DA uptake mechanism. From Exp 1, it could be seen that the acute effect (15 min) of cocaine on striatal metabolism was no longer evident 72 hr later. However, the apparent metabolic state of the nigrostriatal pathway was at a new, lower (~0.3) steady-state in the rats that received repetitive cocaine. In the accumbens, 72 hr following repetitive cocaine administration; DA, DOPAC, and HVA were all diminished by 30% (with no change in the (DOPAC + HVA)/DA ratio). This DA depletion was not evident 15 min following the 21st cocaine administration, suggesting that it takes a period of "withdrawal" to see the change become evident.

Seventy-two hr following the last injection, amphetamine (1 M)-induced "DA-release" was 2.75 ± 0.28 (W-W), 2.69 ± 0.47 (W-C), and 6.07 ± 0.84 (C-C); p<0.05. This data, together with the metabolic data, suggests that repetitive cocaine administration may shift DA from a neuronal pool to a more easily released by amphetamine. Alternatively, there may be changes in the DA carrier or other substrates of the amphetamine release process. (Partially supported by DA-00737).

THE EFFECTS OF 6-HYDROXYDOPAMINE LESIONS OF THE NUCLEUS ACCUMBENS ALTERS THE EFFECTS OF COCAINE ON SCHEDULE-CONTROLLED BEHAVIOR. M. Matthews*, C.S. Sebastian, N.E. Goeders and S.I. Dworkin. Departments of Psychiatry and Pharmacology, Louisiana State University School of Medicine, Shreveport, LA 71130.

Drug self-administration procedures have often been used to investigate central mechanisms of reinforcement. Both specific neurotoxin lesions of discrete brain regions and centrally acting receptor antagonists can alter the rate and pattern of drug intake. Just observed after these manipulations are sometimes suggested to demonstrate a decrease in reinforcing efficacy which implies that the neural systems affected by the manipulation are involved in central mechanisms of reinforcement. However, most drugs have multiple effects on behavior so that specific neurobiological manipulations may also alter some of the unconditioned behavioral effects of the drug. This study determined the effects of cocaine on schedule-controlled behavior before and after 6-hydroxydopamine (6-OHDA) lesions of the nucleus accumbens.

Male F-344 rats were trained on a multiple fixed-interval 4 min, fixed-ratio 15 schedule of food presentation. The presentation dose-response curves for cocaine (3.0-56.0 mg/kg) indicated that cocaine increased responding maintained by the interval schedule but did not change responding maintained by the ratio contingency. While vehicle lesions did not alter the effects of cocaine, bilateral 6-OHDA lesions increased responding maintained by the ratio contingency.

6-OHDA lesions of the nucleus accumbens can alter the unconditioned behavioral effects of cocaine. Therefore, changes in the rate-dependent effects of the drug may be partially responsible for the effects of similar lesions on drug self-administration. Supported by USPHS Grant DA-03011.


The purpose of this study was to test the hypothesis that 6-hydroxydopamine lesions of the nucleus accumbens alter the behavioral pharmacology of cocaine. Metabolic data suggests that repetitive cocaine administration may alter the effects of cocaine. Briefly, following experiments were done. In the first experiment Day 10 pups were individually isolated for 10 min in a warm environment (33°C). Pups were pretreated intraperitoneally with cocaine 2.5 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, or saline 15 min prior to isolation. Ultrasonic vocalizations were monitored through a QMC Rat Detector and recorded. Activity levels were also observed and recorded. Cocaine at doses significantly reduced the number of emitted cries during the entire 10 min of isolation. In fact, compared to the saline controls emitting 240 cries during the 10 min the lowest dose, 2.5 mg/kg cocaine caused a reduction to 77 cries while 5, 10, and 20 mg/kg administered pups were virtually quiet. Pups' activity scores were inversely correlated to their number of cries. That is, the quiet cocaine pups were significantly more active than the saline controls.

In a second experiment saline or cocaine in the above doses was administered to Day 5 rats at the onset of exposure to a novel odor which lasted for 30 min. Some of the pups were administered the opioid antagonist, naltrexone, prior to the cocaine or saline injection. After the odor exposure the pups were returned to their dam. Five days later they were tested for odor preference. A dose-response preference was seen, in that the lowest dose (2.5 mg/kg) and highest dose (20 mg/kg) spent 38% and 26%, respectively, of the test time over the associated odor which does not differ from the control (25%). The 5 mg/kg and 10 mg/kg cocaine-treated pups highly preferred the associated odor choosing to spend 73% and 68%, respectively, in that part of the environment. These preferences were not seen in the naltrexone-cocaine treated pups. Thus, cocaine while producing hyperactivity in the isolated neonate abolishes ultrasonics and can attenuate positive associative learning which is precluded by opioid antagonism.
475.9 CHLORDIAZEPoxide MODIFIES THE INTRAVENous SELF-ADMINISTRATION OF COCAINE IN RATS. K. H. McAllister*, S. M. Dworkin*, S. I. Swerin and N. E. Goeders. (SPON: L. J. Embree) Departments of Pharmacology and Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130

Cocaine is a potent reinforcer that maintains stable patterns of self-administration over long periods of time. Most investigations of the neural substrates mediating the behavioral properties of the drug have focused on catecholaminergic pathways. However, neuropharmacological and receptor binding studies also indicate a complex interaction between cocaine and benzodiazepines. For instance, the co-injection of cocaine and chlordiazepoxide (CDP) increases locomotor activity and injections with saline alone (D'Mello and Stolerman, Br. J. Pharmac., 54:131, 1977). Furthermore, the chronic administration of cocaine alters benzodiazepine receptor density in specific brain regions (Goeders et al., Soc. Neurosci. Abstr., 1987) and these effects may be mediated by dopaminergic mechanisms since depletion of brain dopamine by 6-hydroxydopamine reduces benzodiazepine receptor densities (Sabato et al., Eur. J. Pharmacol., 73:381, 1981). Therefore, it was of interest to investigate whether intravenous cocaine self-administration could be modified by benzodiazepine pretreatment.

Male rats, originally derived from the Fischer 344 strain, were obtained with chronic jugular catheters. The rats were allowed to self-administer cocaine (0.17 mg/0.2 ml infusion) on an FR4:HL 300 sec schedule during 2.5 hr sessions. Completion of the response requirement resulted in a 5 sec infusion of cocaine accompanied by illumination of a house light and onset of the response requirement resulted in a 5 sec infusion of cocaine accompanied by illumination of a house light and onset of a 20 sec tone. When stable patterns of self-administration were obtained, the rats were injected with CDP (0.1-30.0 mg/kg, i.p., 15 min prior to testing) dissolved in physiological saline (which served as the vehicle control).

Injections of 3.0-10.0 mg/kg significantly increased the frequency of cocaine infusions/session, while higher doses (5.6-10.0 mg/kg) decreased the number of infusions. Increasing the cocaine concentration (5.6-10.0 mg/kg) attenuated the CDP-induced reductions in self-administration. The reductions in self-administration of doses of cocaine unaccompanied by increases in the number of limited holds indicating that the effects of the CDP were not likely due to decreases in response rate. These effects of CDP on cocaine self-administration may therefore involve a modification of the stimulus properties or reinforcing efficacy of cocaine. These data provide further support for the involvement of benzodiazepine binding sites in the behavioral effects of cocaine.

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475.10 CONDITIONED PLACE PREFERENCE FOR COCAINE IN RATS: OPEN TEST WITH KNOWN EXPECTED BASELINE CHOICE VALUES. R. C. Mitchell, F. A. Holloway, R. K. Berlin* and J. A. Orad. (SPONSOR: J. A. Holloway) Univ. Oklahoma Health Sciences Center, Oklahoma City, OK 73190

One of the major problems associated with the conditioned place preference (CPP) paradigm in assessing the reward properties of drugs has been marked individual differences in baseline preference for stimuli paired with drug or non-drug conditions. Often, baseline choice behavior is assumed to be random. This study used a new CPP paradigm (V. Vazena & J. Stewart, Neuromolec. Sci., 12:137B, 1986), in order to obtain a known expected choice value for each animal.

The four CPP chambers used in this study were 2 x 2' square with each quadrant floors (rods or wire mesh) which could be arranged in any order. The quadrant floor sections were mounted on a frame attached to contact relays and those signals were fed into a Commodore-64 computer which recorded all latencies and activity. Male, albino rats were randomly assigned to three groups (38/group). On training days, all four quadrants were either rod or mesh. One group received cocaine (COC)/rod and saline (SAL)/mesh pairings (COC+ROD), another COC/mesh and SAL/rod (COC+MES), and the final group was designated as CONTROL. The 50 mg/kg COC or SAL was injected IP 2 min prior to the 20-min training or test sessions. The experiment lasted 3 weeks which permitted 3 test days. Each week, 2 COC and 2 SAL training sessions preceded the no injection TEST day. The COC+rod and COC+mesh groups received conditioning trials each week. On Week 1, one-half the CONTROL rats received COC+rod pairing and the others, COC+mesh pairings; on Week 2, these pairing conditions were reversed; and on Week 3, all CONTROL quadrants were paired with both rod and mesh cues. Each week, on the second COC session, the rats that had been trained to the least preferred quadrant were designated as the baseline value for each animal. At the same time, repeated saline (SAL)/rod pairing was used as the unique quadrant on the test day. The results showed that this unique quadrant significantly (p < 0.05) longer times in the unique CO+ quadrant than on baseline. This trend continued for the COC+rod and COC+mesh groups on Week 2, but not on Week 3 (p > 0.05). The control group displayed a reduced preference for the COC+ quadrant on Week 3 compared to baseline. These results indicate that the unique quadrant throughout the sessions.

Besides demonstrating that stimuli paired with cocaine become "secondary reinforcers," these results provide a refinement of the CPP paradigm by utilizing a known choice value for baseline against which conditioned preferences can be assessed. By selecting the least preferred quadrant during drug sessions as the baseline preference, CPP can be assessed in a conservative fashion against each animal's baseline behavior.

475.11 SENSITIZATION TO COCAINE AND APOMORPHINE: NO INTERACTION IN LESIONED RATS. P.H. Siskos, S. Psychoparmacology, University of Texas Mental Sciences Institute, Houston, TX 77030.

Repeated administration of stimulants often results in a progressively augmented behavioral response. The issue of whether or not such augmentation is mediated for stimulants that have different mechanisms of action has recently been reviewed. It has been suggested that cocaine sensitization is a consequence of the development of a denervation supersensitivity of postsynaptic dopamine (DA) receptors. Apomorphine (APO) sensitization, on the other hand, has been proposed to result from desensitization or normalization of inhibitory presynaptic DA receptors.

A limited test of these suggestions was conducted using the 6-hydroxydopamine (6-OHDA) lesioned rotating rat model. One might expect that rats sensitized to cocaine would show a decreased response to APO relative to their pre-sensitization response, since sensitization of the intact side should make the sides more equally sensitive to APO. Repeated APO administration might be expected to increase in a decrease in rotational response to APO because development of presynaptic hyposensitivity, occurring only on the intact side, should make both sides more equally sensitive to this drug. At the same time, repeated APO should increase cocaine sensitivity because of development of presynaptic hyperactivity on only the intact side.

Male 6-O rats, under pentobarbital anesthesia, were lesioned in the substantia nigra by infusion of 6-OHDA. One group of animals was tested with APO before and after, and again after, 2 weeks of daily cocaine (10 mg/kg) administration. These animals showed a significant increase in rotational response to cocaine, and not to APO. A second group of animals was tested with cocaine before, and again after, 2 weeks of daily APO (0.1 mg/kg) administration. These animals showed a significant increase in rotational response to both cocaine and APO. The results support the hypothesis that the increased rotational response to cocaine can be increased by APO administration.
476.1 A COMPARISON OF THE DISCRIMINATIVE STIMULI OF (+) AND (-) 3,4-METHYLENEDIOXYAMPHETAMINE (MDA) WITH THOSE OF HALLUCINOGENIC AND STIMULANT DRUGS. J. Broadbent, E. K. Michaelson, J. H. Ricke, and J. J. Appel. Behavioral Pharmacology Laboratory, Department of Psychology, University of South Carolina, Columbia, SC 29208.

Due to the reported abuse of the amphetamine derivative 3,4-MDA, the behavioral actions of this compound have been of interest to this laboratory (see abstract by Callahan and Appel). Several reports from other laboratories have indicated that the discriminative stimuli induced by racemic (+) MDA mimic those of (+) amphetamine, LSD and cocaine. Further, the 'stimulant-like' component of the (+) MDA cue appears to due to its (+) isomer while the 'hallucinogenic' component is caused by the (-) isomer (Glennon and Young, 1984). To further test this hypothesis, the two isomers of MDA (+ and -) were given to Carolina, Columbia, SC 29208.

A comparison of the discriminative stimuli of (+) and (-) 3,4-methylenedioxymethamphetamine (MDA) was conducted using a water-reinforced (FR 20) lever-pressing task. Only animals reaching the criteria of 90% correct responding during the completion of the first FR 20 on 10 consecutive days were tested (during extinction sessions). Both (+) and (-) MDA failed to mimic the amphetamine cue at the doses of MDA tested (0.5,1,0.5, and 2 mg/kg). However, animals trained to discriminate cocaine from saline did choose the drug-appropriate lever following both the (+) and, to a lesser extent, the (-) isomer of MDA. In addition both isomers of MDA engendered drug-appropriate responding in animals trained to discriminate LSD from saline.

These data suggest that both isomers of MDA bear at least some similarity to LSD and cocaine. Contrary to previous reports, however, very little similarity of either (+) or (-) MDA to amphetamine was observed at doses tested.


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476.2 DIFFERENCES IN THE STIMULUS PROPERTIES OF 3,4-METHYLENE DIOXYAMPHETAMINE (MDA) AND N-METHYL-1-(3,4-METHYLENEDI0XY AMPHETAMINE) MDMA IN ANIMALS TRAINED TO DISCRIMINATE HALLUCINOGENS FROM SALINE. J. E. Callahan and J. J. Appel. Behavioral Pharmacology Laboratory, Department of Psychology, University of South Carolina, Columbia, SC 29208.

Purportedly, the behavioral effects of (+) MDA (the 'love' drug) have been similar to those of mescaline that are due primarily to its (+) isomer and hallucinogenic effects that are attributed to its (-) isomer. MDMA ('ecstasy'), a structural analog of MDA, appears to have no hallucinogenic properties. In this paper, evidence is presented that questions these conclusions. Two groups of rats were trained to discriminate either LSD (0.08 mg/kg) or mescaline (10.0 mg/kg) from saline in a two-choice, water-reinforced (FR 20) drug discrimination task. When animals in the LSD group (N=1) were tested with (+) MDA (0.5,1.75 mg/kg), responding occurred primarily on the drug-appropriate lever; tests with (-) MDA (0.5,1.75 mg/kg) produced predominantly saline-lever responding. Both isomers of MDMA (0.5,2.0 mg/kg) also engendered responding on the saline lever. In the mescaline group (N=8), (+) MDA (0.5,2.0 mg/kg), (-) MDA (0.5,2.0 mg/kg), (+) MDMA (1.0,2.0 mg/kg), and (-) MDMA (0.5,1.0 mg/kg) mimicked the training drug. Given these results, and the fact that (+) MDA and (+) MDMA have been reported to have stimulant properties, animals in the mescaline group were tested with 0.5,2 mg/kg of amphetamine. No dose of this compound produced drug-like responding.

These results indicate that (+) MDA appears to have effects that are similar to both LSD and mescaline and thus confirm previous findings that (-) MDA is hallucinogenic. Results with the (-) isomer are less easily reconciled with the existing literature. While the discriminable effects of (+) MDA may not resemble those of LSD (at this training dose), they are similar to those of mescaline; therefore, it cannot be concluded that the hallucinogenic potency of MDA is caused solely by the (-) isomer. Since both isomers of MDMA mimic mescaline, this compound does not necessarily lack hallucinogenic effects. It is unlikely that the substitution of MDA and MDMA for mescaline is the result of structural similarities among these compounds since the closely-related compound amphetamine does not have mescaline-like effects.

Supported by USPHS Research Grant R01 DA02543, from the National Institute on Drug Abuse.
476.3 EFFECTS OF MESCALINE ON VISUALLY EVOKED POTENTIALS IN THE AWAKE CAT: AN ANALYSIS OF PRINCIPAL COMPONENTS. D.M. Wilkison, Dept. of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226

In earlier studies we have reported that drugs of abuse differentially alter sensory activity in cortical and thalamic structures. Investigations of the actions of mescaline in chronically prepared, awake cats have been limited due to technical obstacles and our inability to correlate with behavioral actions. Cats, adapted to single restraint, were presented visual (flash), auditory (click) and somatic (electrical stimulation) sensory stimulation on a randomized schedule with probabilities of 20%, 60% and 20%, respectively. Principal component analysis (SAS Institute, Inc.) of visually evoked potentials from six cortical and thalamic sites collected before and after 16 mg/kg mescaline was performed for each cat (n=4). Six factors found common to all experiments were compared. Three factors which were weighted heavily on long-latency, 150-400 msec, components were increased in the visual cortex and medial thalamus while decreased at the anterior suprasylvian cortex. A midlatency component, 40-90 msec, was increased in the suprasylvian cortex. Short-latency factors had variable responses to mescaline.

The data show that mescaline most consistently produced an increase in long-latency responses in the visual cortex evoked potential recorded from cortical and thalamic sites. These potentials appear as phasic or oscillatory waves (5-6 cps) most prominently in intralaminar thalamic. Nucleus reticularis of the thalamus also displayed oscillatory responses to flash after mescaline. Mescaline actions appear to involve a globally projecting system producing slow wave oscillatory cycles in response to flash stimulus. Auditory and somatic stimulation did not produce such activity. This study was supported by NIDA R01 DA 03795.

476.4 ANTAGONISM OF LSD-INDUCED BEHAVIORAL CHANGES IN PRIMATE SOCIAL COLOMIES BY CHRONIC TRAZODONE TREATMENT. R.P. Schlehrner, Jr., C. Kuno*, J.E. Y announcing, R.L. Katt, and J.M. Davis. Dept. of Pharmacodynamics, Univ. of Ill at Chicago and Ill State Psychiatric Inst., Chicago, Ill 60612

Electrophysiological studies have demonstrated that chronic trazodone (TRAZ) administration antagonized the action of LSD on dorsal raphe neurons in the rat (Down and deMontigny, Neurou. Abstr. 16:177, 1986; Prentice and Prentice, in press). The present study examined the effect of acute and chronic administration of TRAZ on LSD-induced behavior in macaque monkeys. Two social colonies of 4 adult Stumptail macaques each served as subjects for the study. The experiment was divided into 3 parts. Following observation of undrugged behavior (BASE), each monkey received (i) acute administration of d-LSD, 0.01 mg/kg, (ii) acute administration of TRAZ, 2 or 4 mg/kg, followed by LSD and (iii) administration of TRAZ, 2 or 4 mg/kg, twice daily for 14 days with LSD given on the last day of treatment. TRAZ was given three hrs. and LSD 15 min. prior to observation. All drugs were injected intramuscularly. The results of this study showed that LSD-induced behavioral changes as previously reported for this species (Schlehrner and Davis, Pharmacol. Biochem. Behav., 25:381, 1986). All monkeys responded similarly to LSD after chronic TRAZ. Treated monkeys could be divided into 3 groups based on appearance and observation data. Three responders appeared essentially unaffected by LSD administration and integrated in colony behavior, 3 monkeys only had a partial response to LSD, while the remaining monkeys were nonresponders and had a full LSD response despite TRAZ treatment. In responders, chronic TRAZ completely antagonized the hallucinogen-specific behavior limb jerks, but more importantly restored vitro postural grooming which had been eliminated by LSD and returned distances scores which were increased by LSD to BASE levels. LSD-induced reductions in self-grooming were antagonized by both acute and chronic TRAZ. Interestingly, LSD-induced body shakes were not antagonized by acute or chronic TRAZ in any monkeys. The results of this study may have important implications for both hallucinogenic and antidepressant drugs. These findings suggest that TRAZ may have utility in the treatment of LSD intoxication. Although these results on responding and nonresponding monkeys remain unresolved at this time, they may be useful in elucidating factors which are responsible for similar results seen with clinical antidepressant drug treatment.

476.5 ANALYSIS OF THE FUNCTIONAL OUTPUT FROM THE SUBSTANIA NIGRA FOLLOWING ACTIVATION BY PHENCYCLIDINE. M.D. Everiist and A. Pert, Biological Psychiatry Branch, NIMH, Bethesda, MD 20892.

We have previously shown that direct unilateral injections of phencyclidine into the substantia nigra of rats produce profound rotational behavior contralateral to the injection. Such rotational behavior may be mediated through several different pathways originating from either the zona compacta or zona reticulata. In order to define these functional outputs which are activated by PCP, rats were implanted with unilateral cannulae aimed for the SN as well as with l.v. jugular catheters. Following recovery, the animals were injected in the SN with 25 nmoles of PCP. Three minutes later, the same rats were also injected l.v. with 100 uc/kg [3H]-2-deoxyglucose (2-DG). Forty five minutes following administration of 2-DG the animals were sacrificed. The brains were prepared for autoradiographic analyses using standard procedures. Injections of PCP into the SN produced rotational behavior in rats which was accompanied by alterations in the metabolic activity of several brain structures. Substantial increases in metabolic activity were found in the caudate nucleus ipsilateral to the injection. Somewhat smaller increases in activity were also seen in the ipsilateral globus pallidus and substantia nigra. No consistent changes could be detected in thalamic nuclei. The SN caudal to the injection site appeared to be profoundly activated. Significant activation could also be detected in the ipsilateral pontine reticular nuclei as well as the medial and lateral olivary complex. While intrapontine PCP appeared to activate nigrothalamic pathways, it is unlikely that these participate in the rotational response since 6-OHDHA lesions on the medial forebrain bundle or semi-laminar nerve to the SN did not alter rotational output elicited by intrapontine PCP. It appears more likely that projections to pontine reticular nuclei are critical since these mid-transitions caudal to the injection were effective in attenuating the effects of intrapontine PCP.

476.6 THE EFFECTS OF SELF-ADMINISTERED PHENCYCLIDINES (PCP) ANALOGUES ON CORTICAL EEG OF THE RAT. L. Marquis, R.P. Quisling, M.G. Webb and J.E. Moreton. Dept. of Pharmacol. & Toxicol., Univ. of Maryland School of Medicine, P.O. Box 240, Baltimore, MD 21203-2400.

The self-administration of PCP is associated with changes in cortical EEG (Marquis et al., Fed. Proc. 46:399, 1987). The present study investigated the changes in EEG produced by the self-administration of the PCP analogues ketamine and 1-(1-phenylcyclohexyl) morpholine (PCM) in the rat. Four female Sprague-Dawley rats implanted with chronic intravenous cannulae and cerebrocortical electrodes were trained to lever press for cocaine (1.0 mg/kg/inj) on a FR10 schedule of reinforcement. Ketamine (3.0 mg/kg/inj) then replaced cocaine in daily 3 hr sessions. Periodically, PCP (0.5 mg/kg/inj), ketamine (4.0) or PCM (4.0 mg/kg/inj) was available for self-administration during a single session while the cortical EEG was time-coded and recorded on a Grass model 7D Polygraph and Vetter Model FM recorder. Lever presses and injections were recorded on the polygraph record beneath the EEG tracings in order to locate sessions of EEG signal for later analysis. Using a Nicolet Pathfinder 1 computer, the minute preceding and following each injection of a test session was digitized. Spectral quantities for frequency, power, mobility and complexity were determined in the 0 to 20 Hz frequency range. Also, relative power in the 0-5 and 5-10 Hz frequency bands was determined. These data indicate that rats will self-administer ketamine and PCM at a rate such that similar effects on cortical EEG are observed when compared to self-administered PCP. Supported by NIDA Grant DA 03717.
Antianxiety drugs such as chlordiazepoxide (CDP) increase punished responding in animals. This antipunishment effect is also commonly seen with CNS depressants (e.g., barbiturates, etorphine). Phencyclidine (PCP) shares antipunishment properties with CNS depressants (Balster & Wessinger, 1983), and it has been shown that CDP increases rates of punished responding in pigeons (Kilpatrick). However, this antipunishment or anticonflict effect has not been shown in rodents. The present study tested rates in a lesion (1966) Conflict test to determine if PCP would produce an increase in punished responding in rodents.

Twenty male rats (B6C3) were trained to respond under a multiple fixed-interval 60-sec (food only) fixed ratio 1 (food + shock) schedule of reinforcement. The components lasted 7 min and 3 min, respectively, with a total session length of 30 min. Initially, the rats were tested with four doses of CDP (0, 2.5, 5.0, and 10.0 mg/kg) administered intraperitoneally (i.p) 30-min preexposure. Then, four doses of PCP (0, 1.0, 2.0, and 4.0 mg/kg) were administered IP 15-min preexposure. Response rates (responses/minute) on test days were analyzed with ANOVAs and Duncan's post hoc tests.

As shown in the table below, both CDP and PCP produced significant increases in punished (P) responding, the high dose of PCP produced a significant decrease; the 2.0 mg/kg dose of CDP produced a significant increase in responding. These results demonstrate that PCP produces an antipunishment effect in rodents similar to that seen in pigeons. It is possible that the abuse of PCP may be in part related to its antipunishment effects.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Punished Resp</th>
<th>Unpunished Resp</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDP</td>
<td>0.49 ± 0.12</td>
<td>1.26 ± 0.45</td>
</tr>
<tr>
<td>PCP</td>
<td>0.25 ± 0.05</td>
<td>0.60 ± 0.05</td>
</tr>
</tbody>
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(Research supported by NIDA grant DA-01442)
476.12 MORPHINE WITHDRAWAL: MODULATION OF AGGRESSION AND MORPHIC ACTIVITY AT DI AND D2 RECEPTORS. E.A. Miczek and J.L. Hohenshil. Dept. of Psychology, Tufts University, Medford, MA 02155.

Substantial and complex neurochemical, physiological and behavioral changes result from chronic exposure to opiates and subsequent withdrawal. Of particular importance appear to be dopamine-mediated functions such as exploratory motor responses and pronounced disturbances of homeostasis. The objective of our studies was to determine whether or not, and how, activation of D1 and D2 receptors selectively modulates certain symptoms of morphine withdrawal, with special attention to heightened aggressive behavior.

Several experiments were designed with mice that were housed as male-female pairs. Before subcutaneous implantation with either placebo or 75-μg morphine pellets, we ascertained that the males exhibited aggressive behavior toward an intruder into their home cage, and we measured their baseline response to pain and rectal temperature. After 3 days of exposure, the no-pellet bag containing the subcutaneous pellet was removed. Five hours after pellet removal physiological and behavioral tests were conducted. This time course was determined previously by measuring the peak frequency of jumps and heightened aggressive behavior as critical indices of morphine withdrawal. During withdrawal from either morphine or placebo pellets, mice were assigned to the following pharmacological challenges: (1) Quisqualate (LTT1155) at either 0.1, 0.3, 0.6, or 1.6 mg/kg, (2) IBMX (60 mg/kg) at either 10, 30, 50, or 100 mg/kg, (3) Combined treatment with quisqualate and SKF83893.

Changes in rectal temperature, tail flick response to a radiant heat stimulus, motor activity (walking, rearing), and aggressive behavior toward intruder males were measured. Videorecords of 5-min observation periods for motor activity and aggressive behavior were analyzed with the aid of a computerized data acquisition system.

Withdrawal from morphine (1) increased attack bites and threats, (2) disrupted walking and rearing while increasing self-directed grooming, (3) lowered body temperature, and (4) led to exploratory behavior. In contrast, the aggressive behavior at doses that did not impair motor activity nor body temperature. By contrast, aggressive behavior was significantly decreased in morphine-withdrawn animals, and led to hypoactivity. In morphine-withdrawn animals, the behavioral and physiological effects of both D1 and D2 agonists were significantly attenuated. If given in combination, both drugs shift the distribution of the motoric elements in the behavioral repertoire of the animals.
76.15 ETHANOL ANTAGONIST Ro 15-4513 IS NOT SELECTIVE FOR ETHANOL. K. T. Britton, L. Percy* and G. F. Koob. VA Med. Center and UCSD Medical School and Alcohol Research Center, SCRF, La Jolla, CA 92037.

Ro 15-4513, an analogue of the benzodiazepine receptor antagonist Ro 15-1788, has been reported to selectively block the anxiolytic and intoxicating properties of ethanol (Sundak, et al., Science, 234, 1243, 1986). We tested Ro 15-4513 in an operant conflict paradigm sensitive to alcohol effects. Ro 15-4513 (0, 1.5, 3.0, 6.0 mg/kg) produced a significant decrease in both punished and unpunished responding in the conflict test. Ethanol (0.75 g/kg) produced a significant release of punished responding that was blocked by pretreatment with 6.0 mg/kg Ro 15-4513. Ro 15-4513 similarly blocked the anticonflict action of pentobarbital (4 mg/kg) and chloridiazepoxide (5 mg/kg).

Rats were also trained on an operant reaction time task involving holding down a lever for 0.25-2.0 seconds to obtain food. Animals treated with 1.5 g/kg of ethanol or 7.5 mg/kg of pentobarbital showed a significant disruption in performance. This disruptive effect was reversed by Ro 15-4513 and FG 7142 in doses of 1.5-6.0 mg/kg. These results suggest that Ro 15-4513 shares many of the inverse agonist properties of the beta carboline FG 7142, and that this inverse agonist activity is the basis for its antagonistic effects against ethanol.

76.16 ETHANOL ANTAGONIST Ro 15-4513: ANXIOTIC EFFECT DEMONSTRATED IN PENTYLENETETRAZOL DISCRIMINATION. C.M. Harris, D. Benjamin*, and H. Lai, Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107.

The imidazobenzodiazepine Ro 15-4513, proposed as a selective ethanol antagonist, blocks the anxiolytic action of ethanol (Sundak et al., Science 234:1243-1247), and produces several direct behavioral and physiological effects opposite to those of anxiolytics. (Bonetti et al., Neurosci. Lett. 18:30, 1984; Miczek and Weerts, Science 235:1127). To test the hypothesis that Ro 15-4513 has a direct anxiogenic action, the drug was tested in the pentylenetetrazol (PTZ) discrimination, a bioassay for anxiogenic drugs. This approach is based on observations that anxiogenic drugs mimic and anxiolytic drugs block the discriminative stimulus produced by PTZ (for description and review, see Lai and Fielding, Drug Dev. Res. 4:3-21, 1984). Rats were trained in a 2-lever food-reinforced operant task to press one lever after injection of PTZ, and the other lever after saline. They were utilized in drug tests in which the percentage of rats selecting the PTZ-appropriate lever served as a measure of the intensity of PTZ-like stimuli. Rats selected the PTZ-lever in a dose-dependent manner after Ro 15-4513 (ED50 = 2.1 mg/kg), indicating a PTZ-like subjective effect. Behavioral toxicity was not evident; convulsions, tremors, or rigidity did not occur at doses of Ro 15-4513 up to 40 mg/kg, and the X rats completing the lever-pressing task was not significantly reduced at any dose. The PTZ-like stimulus of Ro 15-4513 was antagonized in a dose-dependent manner by pretreatment with the anxiolytic drug diazepam (ED50 = 9.0 mg/kg), further indicating the PTZ-like quality of the Ro 15-4513 stimulus. The PTZ-like stimulus produced by Ro 15-4513 was blocked by the benzodiazepine receptor antagonist Ro 15-1788.

These data support the hypothesis that Ro 15-4513 is anxiogenic and that its effect is mediated by its action at the benzodiazepine receptor. (Ro 15-4513, Ro 15-1788 and diazepam were supplied free of charge by the Hoffmann-La Roche Company; the study was supported by grant # ROI-AA06890 from NIAAA.)